

STUDY PROTOCOL

Rwanda WISH Study (Women's Improvement of Sexual and reproductive Health)

Sponsor	University of Liverpool
Study site	Rinda Ubuzima KN 50 th Avenue (formerly Rue Akagera 716), Kiyovu, Kigali, Rwanda P.O. Box 4560, Kigali, Rwanda Tel: +250-252-503431 Fax: +250-252-503389
Chief investigator	Professor Janneke van de Wijgert, PhD MPH MSc Institute of Infection and Global Health, University of Liverpool Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE Tel office: +44-151-795-9613; Tel mobile: +44-755-719-5108 Email: j.vandewijgert@liverpool.ac.uk
Principal Investigator	Stephen Agaba, MD Rinda Ubuzima (see contact details above) Tel mobile: +250-788 357 866 Email: agabakarema@yahoo.co.uk
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Chief Investigator

Janneke van de Wijgert

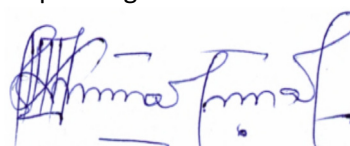


Signature

Date: 6 June 2016

Principal Investigator

Stephen Agaba



Signature

Date: 6 June 2016

STATEMENT OF PROTOCOL COMPLIANCE AND CONFIDENTIALITY

By signing this statement, the Principal Investigator agrees to:

- Conduct the present study in full accordance with the most recent approved version of the protocol, within applicable timelines, according to the relevant standard operating procedures (SOPs) and in full agreement with all applicable regulations and the international guidelines regarding the conduct of clinical research.
- Permit study-related monitoring, audits, independent ethics committee review, and regulatory inspections, providing direct access to source data/documents during and after the course of the study.
- Make the protocol and all relevant related study documentation available to all physicians, nurse/counsellors, laboratory staff and other personnel who participate in conducting this study. Ensure that the study team receives adequate training so that they are fully informed and qualified to conduct the study.
- Maintain all study documentation in accordance with the International Conference on Harmonisation Good Clinical Practice and other relevant guidelines or until the Chief Investigator stipulates in writing that maintenance is no longer required.

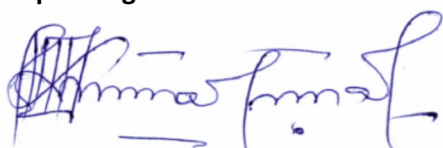
The Principal Investigator also acknowledges that this protocol contains confidential information. As such, the information may not be disclosed to anyone except persons involved in implementation of the study, relevant ethics committees and relevant drug regulatory authorities, unless specific permission is granted in writing by the Chief Investigator, or disclosure is required by relevant laws and regulations.

The Principal Investigator:

- Authorises the Chief Investigator and her team at the University of Liverpool, Liverpool, UK, to have full access to all data pertinent to this study and to receive and test study specimens as described in detail in this protocol.
- Understands that copies of all computerised databases will be kept and fully accessible by the study team at the UoL, the study team at Rinda Ubuzima, as well as third parties who have been given permission by the Chief Investigator and the Principal Investigator.
- Acknowledges that scientific publications and other planned uses of the study data and study specimens will be governed by a separate data and specimens sharing policy, and will adhere to the agreements set forth in that policy.

Principal Investigator:

Stephen Agaba



6 June 2016

Signature

Date

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LIST OF ACRONYMS

ART	Antiretroviral therapy
ASSURED	Affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users
BV	Bacterial vaginosis
(e)CRF	(electronic) Case report form
CT	<i>Chlamydia trachomatis</i>
DALY	Disability adjusted life years
EDCTP	European and Developing Countries Clinical Trials Partnership
GBP	Great Britain Pound
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV-2	Herpes simplex virus type 2
ICF	Informed consent form
ICH	International Conference on Harmonization
IDI	In-depth interview
IGH	Institute of Infection and Global Health, UoL, Liverpool, UK
ITM	Institute of Tropical Medicine, Antwerp, Belgium
LGV	Lymphogranuloma venereum
M&E	Monitoring and evaluation
NG	<i>Neisseria gonorrhoea</i>
NPV	Negative predictive value
PCR	Polymerase chain reaction
(e)PIR	(electronic) participant identification register
PIS	Participant information sheet
POC(T)	Point of care (test)
PPV	Positive predictive value
RU	Rinda Ubuzima
SOP	Standard operating procedure
SRH	Sexual and reproductive health
STI	Sexually transmitted infection
TV	<i>Trichomonas vaginalis</i>
UoL	University of Liverpool, Liverpool, UK
UK	United Kingdom
USA	United States of America
UTI	Urinary tract infection
VCT	Voluntary counselling and testing
WHO	World Health Organisation

1. PROTOCOL TEAM ROSTER

Rinda Ubuzima (RU), Kigali, Rwanda	Study role	E-mail address
Stephen Agaba, MD	Principal Investigator	agabakarema@yahoo.co.uk
Gilles Ndayisaba, MD	Co-Investigator (clinical)	ndayisaba@gmail.com
Mireille Uwineza, BSc	Study coordinator	uwinezamireille@yahoo.fr
Marie Michelle Umulisa, MA	Coordinator recruitment, outreach, and social science	mariemichelleu@yahoo.fr
Lambert Mwambarangwe, BSc	Laboratory coordinator	mwambara2@yahoo.fr
Vincent Karangwa	Administrative oversight at RU	karavinc@yahoo.fr
University of Liverpool (UoL), Liverpool, UK	Study role	Email address
Janneke van de Wijgert, MD PhD MPH	Chief Investigator	j.vandewijgert@liverpool.ac.uk
Marijn Verwijs, MD	Project Manager	marijnverwijs@gmail.com
Institute of Tropical Medicine (ITM), Antwerp, Belgium	Study role	Email address
Vicky Jespers, MD PhD MEpi	Co-Investigator (clinical)	vjespers@itg.be
Tania Crucitti, PhD PharmD	Co-investigator (laboratory)	tcrucitti@itg.be
Thérèse Delvaux, MD PhD	Co-investigator (social science)	tdelvaux@itg.be

2. SYNOPSIS

Purpose	<p>The current standard of care for urogenital infections in Rwanda is syndromic management. Many urogenital infections are asymptomatic and therefore completely missed, and the management of vaginal discharge syndrome is known to be suboptimal. The primary objective of this study is to evaluate whether it is feasible to improve urogenital infection care in high risk women in Kigali, Rwanda (see 'study population' below), using point of care (POC) diagnostic testing for HIV, <i>Trichomonas vaginalis</i> (TV), and bacterial vaginosis (BV) in all women; POC testing for <i>Neisseria gonorrhoeae</i> (NG), <i>Chlamydia trachomatis</i> (CT), and syphilis in pregnant women and women assessed to be at high risk for these infections using a risk scoring questionnaire; and management of vaginal candidiasis, urinary tract infection (UTI), genital ulcers/inguinal bubos, and lower abdominal pain in women reporting relevant symptoms. The secondary objectives of this study are 1) to evaluate the performance and 2) cost effectiveness of the POC tests for CT/NG, TV and BV; and 3) to obtain the opinions of stakeholders (clinicians, programme implementers and policymakers) about 3a) the potential improvement of urogenital infection care in Rwanda as well as 3b) the potential roll-out of novel vaginal microbicides and multipurpose prevention technologies for HIV and pregnancy prevention as soon as efficacious products become available (to prepare for future projects at Rinda Ubuzima (RU)).</p>
Study Design	<ol style="list-style-type: none"> 1. This is a cross-sectional study. The improved urogenital infection care services will be advertised to women in Kigali, Rwanda, via RU's existing networks, targeting women with urogenital complaints as well as women without urogenital complaints who have had high risk behaviour. The services will be available for free at RU's research clinic for the duration of the project. All consenting women who attend RU's research clinic during the study period will be offered: <ol style="list-style-type: none"> a. Voluntary counselling and testing (VCT) for HIV. b. Urine pregnancy test if indicated and contraception counselling. c. POC testing for UTI if UTI symptoms are present. d. POC testing for TV and BV regardless of symptoms, and management of vaginal candidiasis based on symptom-reporting. e. POC testing for syphilis and CT/NG if pregnant or considered at risk by risk scoring questionnaire. f. Syndromic management of genital ulcers/inguinal bubos and lower abdominal pain. g. Treatment and partner notification and treatment as appropriate, and referrals to antenatal, family planning, HIV and cervical cancer screening care. <p>Information about sociodemographics, risk behaviour, sexual and reproductive health history and current urogenital symptoms will be collected during the clinic visit. Women can opt out of each service offered. Services will be delivered within one half day. However, women can choose to leave before all results are available, and be contacted by study staff when results are available, which is particularly relevant for women undergoing CT/NG POC testing (which takes about 90 minutes).</p> 2. Vaginal swabs for storage will be taken from all consenting women (women can choose between self- or clinician-sampling) for additional research testing at the end of the study to allow for performance evaluation of the

	CT/NG, TV and BV POC tests. 3. Opinions of stakeholders will be gathered during workshops (one before and one after completion of the study) and in-depth interviews (IDIs).
Study Site	Rinda Ubuzima (RU) research clinic, Kigali, Rwanda
Study Products	None
POC Diagnostic Tests	<ul style="list-style-type: none"> • HIV: POC tests and testing algorithm currently approved by the Rwanda Ministry of Health, locally available • Pregnancy: Validated urine human chorionic gonadotropin (hCG) test, locally available • UTI: Validated urinalysis test, locally available • BV: Ecocare Comfort Vaginal pH swab (Merete Medical, Luckenwalde, Germany), to be imported • TV: OSOM POCT (Sekisui Diagnostics, San Diego, CA, USA), to be imported • CT/NG: GeneXpert (Cepheid, Sunnyvale, CA, USA), to be imported • Syphilis: Alere Determine POCT (Alere International, Galway, Ireland), regionally available
Study Population	Adult women living in the city of Kigali who are at high risk of HIV/urogenital infections (defined as having had more than one sexual partner in the last 12 months OR having been treated for a sexually transmitted infection (STI) in the last 12 months) regardless of the presence of current urogenital symptoms. Women who are known to be HIV-positive and/or pregnant will not be excluded.
Study Duration	One year
Study Endpoints	<ul style="list-style-type: none"> • Primary: Monitoring and Evaluation (M&E) indicators of the services offered (such as numbers of women counselled and risk scored, diagnostic tests conducted, infections diagnosed and treated, referrals made, etc.), and responses to a client satisfaction survey and staff survey about their experiences with the services. • Secondary: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for 1) syndromic management, 2) risk scoring followed by POC testing (CT/NG), and 3) POC testing of everyone (CT/NG, TV and BV). • Secondary: Cost-effectiveness parameters (such as costs for each diagnostic algorithm, costs per correctly and incorrectly managed infection, and total cost per disability adjusted life year (DALY) averted) • Secondary: Consensus, majority and minority opinions of stakeholders from transcripts of workshops and IDIs.

3. INTRODUCTION

3.1 Background

Rinda Ubuzima (RU) in Kigali, Rwanda, is a non-governmental organisation specialised in HIV prevention and sexual and reproductive health (SRH) research in women. RU has operated a research clinic and laboratory in Kigali since 2004, and has successfully implemented 9 large SRH studies (1-10). The main purpose of this study is to use the capacity that RU has built to evaluate whether it is feasible and affordable to improve SRH services, and particularly the diagnosis and treatment of urogenital infections, in high risk women.

Urogenital infections in different female populations at risk in Rwanda

In the context of the above-mentioned SRH studies, RU has worked with high risk women (most of whom were sex workers), female clients of public voluntary HIV counselling and testing (VCT) sites, HIV-positive women before and during antiretroviral therapy (ART) at public clinics, and women in the general population. The following table, containing data from these studies, shows that all urogenital infections are common in these populations.

Prevalence (%)	KHIS high risk women ¹	KHIS female VCT clients ²	VBMS HIV-neg high risk ¹	VBMS HIV-pos women ³	SEARCH HIV-pos ³ Pre-ART/on ART
Year of the Study	2006-2009	2007-2009	2010-2012	2010-2012	2007-2010
HIV	24	13	0 ⁴	100 ⁴	100 ⁴
HSV-2	60	43	47	83	86 / 92
High risk HPV	37	ND	ND	ND	38 / 53
Syphilis	7	ND	7	20	3 / 4
<i>Neisseria gonorrhoeae</i>	12	ND	7	13	14 / 7
<i>Chlamydia trachomatis</i>	5	ND	10	0	5 / 5
<i>Trichomonas vaginalis</i>	17	ND	10	10	ND
Bacterial vaginosis (Nugent 7-10)	40	ND	44	36	ND
Intermediate microbiota (Nugent 4-6)	15	ND	19	21	ND
Vaginal candidiasis	7	ND	10	13	ND
Urinary tract infection	ND	ND	23	23	ND
Uses modern method of contraception excl. condoms	19	14	70	54	40
Never HIV tested before	35	25	ND	NA	NA

KHIS [1-4], VBMS [5-7] and SEARCH [8] are study acronyms; ND=not done; NA=not applicable.

1. Female sex workers or other women with multiple partners.
2. Clients of public VCT centres in Kigali city; not selected by risk behaviour.
3. HIV positive women receiving care at a public HIV clinic in Kigali; not selected by risk behaviour.
4. By study design; only HIV-negative or HIV-positive women included.

The viral sexually transmitted infections (STIs) herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV) were the most common, followed by the various vaginal infections (bacterial vaginosis (BV) and intermediate microbiota, *Trichomonas vaginalis* (TV), and vaginal candidiasis) and urinary tract infections (UTIs). The bacterial STIs *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), and syphilis were less common, but higher prevalence rates were measured in a currently ongoing study in high risk women at RU: 12% for NG and 20% for CT (unpublished data).

Sequelae of urogenital infections

If left untreated, urogenital infections are associated with many devastating sequelae, particularly in women (11). NG, CT and BV are associated with infertility and pelvic inflammatory disease, and in pregnant women with miscarriage and preterm birth. Syphilis causes neurological symptoms, and in pregnant women congenital syphilis. UTIs can progress to kidney infection and sepsis. High risk HPV viruses are associated with the development of cervical, anal and head-and-neck cancers.

Furthermore, it has become increasingly clear that the HIV and urogenital infection epidemics fuel one another (12, 13). Therefore, the admirable gains made in HIV prevention and care in Rwanda in the last decade (14), are threatened by the fact that urogenital infections have not yet been brought under control.

With the increasing use of highly sensitive and specific molecular diagnostic tests for various urogenital infections in research studies, it has become clear that asymptomatic infections are also associated with the above-mentioned adverse outcomes, sometimes to the same extent as symptomatic infections (7, 15). Furthermore, a considerable body of research has now shown that the majority of urogenital infections in women are asymptomatic (11). In addition to promptly identifying and treating symptomatic women, screening of asymptomatic women at high risk may therefore be necessary to reduce the prevalence of urogenital infections and their sequelae (11).

Syndromic management

The high burden of STIs worldwide, but particularly in sub-Saharan Africa, has long been recognised by the World Health Organisation (WHO). In an effort to control STIs in resource-poor settings with no or limited laboratory infrastructure, the syndromic management guidelines were launched in 1978, and updated in 2003 (16) and 2008 (17). These guidelines categorise STIs by the symptoms they cause, and call for treatment of all pathogens that might cause that syndrome. The current WHO syndromic guidelines for women are as follows (16, 17):

- Vaginal discharge syndrome without lower abdominal pain: Treat for BV and TV. If the discharge is curd-like and erythema and itching are present, also treat for vaginal candidiasis. If the NG and CT prevalence is high, or the individual's risk for these infections is considered high, also treat for NG and CT.
- Lower abdominal pain (with or without discharge): Requires abdominal and pelvic examination. If cervical motion or adnexal tenderness is present: treat for pelvic inflammatory disease. Refer to the hospital in case of a missed period, recent delivery/abortion/miscarriage, abdominal guarding or rebound tenderness, abnormal vaginal bleeding, or abdominal mass.
- Genital ulcers: Treat for syphilis and HSV-2. Also treat for chancroid, lymphogranuloma venereum (LGV) and granuloma inguinale where these infections are prevalent.
- Inguinal bubos without genital ulcers: Treat for LGV and chancroid.
- Inguinal bubos with genital ulcers: Follow the genital ulcers guideline.

The diagnosis and treatment of UTIs is not included in the WHO syndromic management guidelines, but in daily practice women who present with UTI-associated complaints are usually treated.

The obvious advantage of the syndromic approach is that no laboratory testing is needed but the obvious disadvantage is that all asymptomatic infections are missed. As mentioned earlier, the majority of urogenital infections in women are asymptomatic. Furthermore, research has shown that the algorithm for vaginal discharge syndrome in women has low sensitivity and specificity (18, 19). Our own research at RU confirms this (data not yet published). In contrast, research has shown that syndromic management of genital ulcers/inguinal bubos is adequate for both men and women (17), and that UTIs can be adequately managed based on urinalysis in patients with self-reported UTI-associated symptoms (20; although this is understudied in resource poor settings).

Point of care (POC) diagnostic tests

One way to improve urogenital infection care in women is to introduce POC diagnostic testing (21). Validated POC tests for HIV (22), syphilis (23), UTIs and pregnancy have been available for many years and are well-integrated into public clinics, including in resource poor settings. Most POC tests for HIV and syphilis are immunochromatographic assays that detect pathogen antigens or antibodies in small amounts of blood (a finger prick is sufficient). UTI and pregnancy POC tests are dipsticks that are submerged in a urine sample. UTI dipsticks detect leukocytes (among other analytes), and pregnancy dipsticks detect the human chorionic gonadotropin (hCG) hormone.

More recently, POC tests were developed for TV and CT/NG. The OSOM TV POC test (Sekisui Diagnostics, San Diego, CA, USA) is an immunochromatographic dipstick test that detects TV antigens in vaginal swab eluent; it was found to be 77% sensitive and 99% specific compared to TV polymerase chain reaction (PCR) in a study in Brazil and South Africa (24). This means that 24% of cases were misdiagnosed (23% false-negative and 1% false-positive). In Rwanda, TV is currently diagnosed by symptoms only, and a recent study in India found that 86% of patients with PCR-proven TV were misdiagnosed on the basis of symptoms alone (25). The TV OSOM POCT is therefore a significant improvement over the current situation.

The development of a POC enzyme immunoassay for NG and CT has proved difficult: all tests that were developed and evaluated thus far had poor sensitivity (26, 27). However, a POC real-time molecular test (detecting NG and CT DNA) is now available: the GeneXpert test (Cepheid, Sunnyvale, CA, USA) (28). While the GeneXpert test is highly sensitive and specific for NG and CT, it does not fulfil all ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users) criteria (27): for example, testing requires a machine and about 90 minutes per sample. Research on CT/NG POC tests that do fulfil the ASSURED criteria is ongoing. Meanwhile, the GeneXpert platform is a significant improvement over syndromic management (the above-mentioned recent study in India found that 80% of women with PCR-proven CT and 50% of women with PCR-proven NG were misdiagnosed on the basis of symptoms alone; 25) and traditional PCR platforms, and is being rolled out in select specialised centres across Africa.

BV is usually diagnosed empirically (based on self-reported symptoms) in public primary care clinics, by Amsel criteria in specialised clinics (29), and by Gram stain Nugent scoring in specialised referral clinics and research settings (30). The Amsel criteria and Nugent scoring require skilled microscopists, who are usually not available in resource-poor settings. In the last decade, entire bacterial communities in the vagina have been characterised in detail (referred to as 'vaginal microbiota') using novel molecular testing techniques. We now know that vaginal microbiota that are dominated by *Lactobacillus* species are healthy and that BV is a polybacterial dysbiosis: lactobacilli are no longer dominant and the bacterial diversity in the vagina increases (31). Intermediate microbiota might represent a transition to or from dysbiosis. RU studies showed that increasing bacterial diversity in the vagina is not associated with the presence of urogenital symptoms, but is associated with increasing prevalence of HIV, HSV-2, HPV, bacterial STIs, and UTIs (15). While more research is needed to investigate these relationships, we believe that the currently available evidence is sufficient to justify treatment of both symptomatic and asymptomatic BV. Our research has also shown that the vaginal pH (which can be easily measured by putting some vaginal fluid on litmus paper in the vaginal pH range) is strongly correlated with the types of bacteria that are present in the vagina. Lactobacilli are very strong lactic acid producers and cause the vagina to be acidic (low pH, typically 3.6-4.1) whereas the bacteria associated with BV produce no or less lactic acid and cause the vaginal pH to be higher (31). Based on data from our previous studies in similar populations, we have chosen to treat all women with a vaginal pH ≥ 5.0 regardless of symptoms for BV. This cut-off achieved a sensitivity of 71% and a specificity of 76% when a Nugent score of 7-10 was used as the gold-standard for BV diagnosis in unpublished studies conducted at RU. In addition,

women with a lower vaginal pH will be treated when they have severe symptoms consistent with BV to minimise false-negative diagnoses. In comparison, a South African study in 2012 showed that the sensitivity of using symptoms alone to diagnose BV was only 10% (32).

Integration of POC tests into urogenital infection care

In this study, we will offer POC testing for HIV, TV and BV to all women, on a voluntary basis regardless of the presence of symptoms. As is clear from the table above, these infections are extremely common in high risk women in Kigali, which justifies testing of all women. Furthermore, validated, user-friendly and affordable POC tests for these infections are available, TV and BV are curable, and women testing HIV-positive will benefit from referral to clinics providing antiretroviral therapy. Vaginal candidiasis and UTIs are also common but these infections typically become symptomatic when the fungal/bacterial load increases, and are easily diagnosed based on symptoms. In this study, we will treat vaginal candidiasis based on symptoms alone, and we will test women with UTI symptoms by urinalysis dipstick test to determine whether UTI treatment is required.

The curable STIs NG, CT and syphilis are less common, and the POC test for CT/NG is time-consuming and expensive. For these reasons, we will use a risk scoring questionnaire to determine which women are at sufficiently high risk to warrant POC testing. Risk scoring questionnaires were shown to be sensitive and specific in some studies (19, 33). To maximise the performance of the risk scoring questionnaire to be used in this study, we will use data collected at RU in previous studies to design the questionnaire. Pregnant women will be tested for NG, CT and syphilis regardless of their risk score because of the potential consequences of these curable STIs on the pregnancy and neonate.

While HSV-2 and HPV are also very common, these infections cannot be cured and do not require lifelong therapy. Instead, we will offer acyclovir treatment for active HSV2 outbreaks (when genital vesicles are present) as part of syndromic management of genital ulcers. HPV testing and vaccination is currently not available in Rwanda for our study population of adult high risk women (34). However, we will refer women to cervical cancer screening programs available elsewhere in Kigali.

3.2 Rationale

The primary objective of this study is to evaluate whether it is feasible to improve urogenital infection care in high risk women in Kigali, Rwanda, using POC diagnostic testing for HIV, TV, and BV in all women; POC testing for CT/NG, and syphilis in pregnant women and women assessed to be at high risk for these infections using a risk scoring questionnaire; and management of vaginal candidiasis, UTI, genital ulcers/inguinal bubos, and lower abdominal pain in women reporting relevant symptoms. The secondary objectives of this study are 1) to evaluate the performance and 2) cost effectiveness of the POC tests for CT/NG, TV and BV; and 3) to obtain the opinions of stakeholders (clinicians, programme implementers and policymakers) about 3a) the potential improvement of urogenital infection care in Rwanda as well as 3b) the potential roll-out of novel vaginal microbicides and multipurpose prevention technologies for HIV and pregnancy prevention as soon as efficacious products become available (to prepare for future projects at RU).

4. STUDY DESIGN, OBJECTIVES AND ENDPOINTS

4.1 Study design

We will conduct a year-long cross-sectional study in RU's research clinic, during which RU staff will routinely offer improved urogenital care services to all eligible high risk women who attend the clinic. The improved services will be advertised via RU's existing network of community mobilisers and via word of mouth. The advertising will clearly state that the services are: 1) for women with

urogenital complaints as well as women without urogenital complaints who have had high risk behaviour; 2) at no cost to them; but are 3) only temporarily available for the duration of the project; and 4) only available once per participant (this includes one urogenital infection screening visit plus any additional follow-up that is required to manage diagnosed infections).

During the informed consent process, each service will be explained, and women will be told that they can opt out of each service offered. Our aim is to conduct all services (including provision of treatment) within one half day (the Main Visit) to minimise loss to follow-up. However, women can choose to leave before all results are available, and be contacted by study staff when results are available (see section 5.4 for a description of how women will be contacted), which is particularly relevant for women undergoing CT/NG POC testing (which takes about 90 minutes).

Services to be offered (see also section 6: study procedures and appendix 14.2: WISH clinical algorithms; all procedures will be implemented in accordance with existing RU SOPs):

1. *VCT for HIV*. Women who are known HIV-positive but attend the clinic to be screened for urogenital infections can skip the HIV testing step. Women who do not know their HIV status, but do not want to be tested, can also skip the HIV testing step but will be strongly encouraged to get tested. The RU clinicians and on-site laboratory have much experience with HIV counselling and testing in accordance with the national testing algorithm that was approved by the Rwanda Ministry of Health. All women regardless of HIV status, and whether or not they choose to be tested, will be educated about HIV prevention options available in Kigali.
2. *Urine pregnancy test* if indicated and contraception counselling.
3. *POC testing for UTI*. Women will be asked about symptoms typically associated with UTIs; their urine will be tested by urinalysis strip if symptoms are reported; and treatment will be given as appropriate.
4. *POC testing for vaginal infections regardless of symptoms*. All women regardless of symptoms will be offered vaginal pH measurement with litmus paper attached to a vaginal swab to diagnose BV, POC testing for TV, and treatment for BV and/or TV as appropriate. They will also be asked about symptoms typically associated with vaginal candidiasis, and treatment based on symptom-reporting. In addition, all women will be counselled about safer sex, cessation of harmful vaginal hygiene practices, and male penile hygiene, to minimise BV recurrence.
5. *POC testing for other curable STIs (syphilis, NG and CT) if pregnant or considered at risk by risk scoring questionnaire*. The questionnaire will be developed in the beginning of the project using the behavioural factors that were most strongly associated with these curable STIs in previous RU studies. If considered at high risk by risk scoring questionnaire or pregnant, POC testing will be conducted, and treatment given as appropriate.
6. *Treatment of genital ulcers/inguinal bubos and lower abdominal pain according to the syndromic management guidelines*.
7. *Partner notification and treatment, if applicable*.
8. *Referrals to antenatal, HIV, family planning and other care, if applicable*.

In addition to offering these services, research activities will be carried out as follows:

1. To monitor and evaluate the services offered, we will collect data on monitoring and evaluation (M&E) indicators. Data for most M&E indicators will be available as part of routine clinic procedures (such as numbers of women counselled and risk scored, diagnostic tests conducted, infections diagnosed and treated, referrals made, etc.) but others will require interviewing participants at the end of their visit (client satisfaction survey), interviewing staff at the end of the study (about their experiences providing the services), timing of procedures done by different staff members, and timing of client trajectories.
2. To evaluate the performance of POC tests for NG, CT and TV compared to providing syndromic management alone, we will ask all women at the beginning of their visit (before any POC tests

have been done) whether they have urogenital complaints. We will ask them these questions exactly in the same manner as we would have done if we would only have offered syndromic management. In addition, we will ask all women to collect two vaginal swabs via self- or clinician-sampling (the woman's own choice) for temporary storage and testing for research purposes after the client has left the clinic. Stored swabs from all women who did not undergo GeneXpert CT/NG POC testing during their Main Visit will be tested by GeneXpert soon after their Main Visit so that we have a GeneXpert result for all women. If funding permits, we will also test stored swabs from all women by molecular tests for TV, BV and UTI after completion of the study (within two years after termination of data collection). This will allow for sensitivity, specificity, PPV, NPV and cost effectiveness comparisons of syndromic management, POC testing after risk scoring, and POC testing of all women, and will improve our understanding of organisms associated with BV and UTI. If we identify any NG or CT infections by GeneXpert that had not been treated at the Main Visit, we will ask these women to return to the study clinic for repeat testing and treatment if applicable.

3. To evaluate the cost effectiveness of introducing POC tests, we will collect data on cost-effectiveness parameters (such as total costs for each diagnostic algorithm, costs per correctly and incorrectly managed infection, and total cost per DALY averted).
4. We will conduct qualitative research with stakeholders (clinicians, programme implementers and policymakers) regarding the potential improvement of urogenital infection care in high risk women, and also the potential roll-out of novel vaginal microbicides and multipurpose prevention technologies for HIV and pregnancy prevention as soon as efficacious products become available (to prepare for future RU projects). The opinions of stakeholders will be solicited during two workshops (one to be held before the start of the study and one after completion of the study) as well as in about 10 individual IDIs.

4.2 Specific objectives and endpoints

<u>Primary objectives</u>	<u>Primary endpoints</u>
1. Implement improved urogenital infection care services and monitor and evaluate these services	<ul style="list-style-type: none"> • M&E indicators, such as: <ul style="list-style-type: none"> – Inputs: Procurement experiences, infrastructure/training requirements; – Activities/outputs: Numbers of clients counselled and risk scored, diagnostic tests conducted, infections diagnosed and treated, and referrals made; staff time required for each activity; time spent at the clinic for each client trajectory; – Outcomes/impacts: Improvements of access to and quality of services (from client satisfaction survey, staff interviews).
<u>Secondary objectives</u>	<u>Secondary endpoints</u>
1. Compare performance of syndromic management, POC testing after risk scoring, and POC testing of everyone for the diagnosis of NG, CT, TV, and BV.	<ul style="list-style-type: none"> • Sensitivity, specificity, PPV, and NPV for: <ul style="list-style-type: none"> – Syndromic vs POC testing of everyone (NG, CT, TV and BV) – Syndromic vs risk scoring followed by POC testing (NG, CT) – Risk scoring followed by POC testing vs POC testing of everyone (NG, CT) – If funding permits, also all of the above vs gold standard molecular testing of everyone (NG, CT, TV, BV, and UTI)
2. Compare cost-effectiveness of all of the above	<ul style="list-style-type: none"> • Cost effectiveness parameters, such as: <ul style="list-style-type: none"> – Total costs for each diagnostic algorithm – Costs per correctly and incorrectly managed infection – Total cost per disability adjusted life year (DALY) averted
<u>Qualitative research objectives</u>	<u>Qualitative research endpoints</u>
3a. Obtain stakeholder opinions about potential	<ul style="list-style-type: none"> • Consensus, majority and minority

improvement of urogenital infection care in high risk women, as evaluated in the cross-sectional study.	opinions of stakeholders from transcripts of workshops and IDIs.
3b. Obtain stakeholder opinions about the potential roll-out of novel vaginal microbicides and multipurpose prevention technologies for HIV and pregnancy prevention as soon as efficacious products become available (to prepare for future RU projects).	<ul style="list-style-type: none"> • Consensus, majority and minority opinions of stakeholders from transcripts of workshops and IDIs.

4.3 Sample size

The primary objective is descriptive. Formal sample size calculations were therefore not performed. We estimated to be able to see 500-700 women in one year with the funding we received from EDCTP. However, our estimate may have been conservative, in which case the sample size may be increased to up to 1,000 women. Based on prevalence data from recent RU studies in the same high risk population, if we see 500 clients (assuming that they do not opt out of any services offered), we expect to diagnose 100 cases of HIV, 35 cases of syphilis, 60 cases of NG, 75 cases of CT, 75 cases of TV, 175 cases of BV, 50 cases of vaginal candidiasis, and 115 cases of UTI.

Data for the qualitative research objectives will be gathered at two workshops with stakeholders: one to be held before initiation of the study and one after completion of the study. In addition, IDIs will be done with clinicians, programme implementers and policymakers. The number of IDIs to be held will be governed by data saturation: no new interviews will be done when they no longer generate new information. We expect to conduct approximately 10 IDIs.

4.4 Randomisation and blinding

There will be no randomisation and blinding in this study.

5. STUDY POPULATION

5.1 Target population

Our target population is adult women living in the city of Kigali who are at high risk of HIV/STIs. In this study, high risk will be defined as having had more than one sexual partner in the last 12 months OR having been treated for an STI in the last 12 months. Women who are known to be HIV-positive and/or pregnant will not be excluded from participation. Women will be allowed to participate regardless of whether they currently have urogenital symptoms.

5.2 Eligibility criteria

Inclusion criteria:

- Female, at least 18 years old (no upper age limit)
- At high risk of HIV/STIs, defined as having had more than one sexual partner in the last 12 months OR having been treated for an STI in the last 12 months
- Willing and able to provide written informed consent. According to the Rwandan ethics guidelines, unmarried women aged 18-20 also need the written informed consent of a parent or guardian.

Exclusion criteria:

- Already participated in this study before (each woman can only participate once)
- Participating in another health intervention study
- For any other reason as judged by the Principal Investigator (these reasons will be recorded)

5.3 Recruitment

RU will use its network of community mobilisers to advertise the services in the Kigali neighbourhoods where recruitment for previous RU studies with high risk women took place. The community mobilisers are high risk women themselves who have participated in RU projects in the past; RU has worked with them for several years. Advertisement may include handing out flyers and RU staff attending community meetings to explain the project to relevant communities. We also expect to reach women via word-of-mouth. Recruitment will not start until permission has been obtained from the local authorities in those neighbourhoods. Standard RU procedures will be used to ensure that confidentiality is not breached. For example, the project vehicles do not carry a logo and outreach staff do not wear uniforms. RU staff may check potential eligibility of individual women who are interested in participating using an anonymous checklist. At this stage, no individual data will be recorded. Women who are interested and eligible will be invited to attend the RU research clinic in Kiyovu, Kigali.

5.4 Retention and withdrawals

All participants will be asked to provide contact information for herself and at least one other contact person. RU's preferred way of contacting participants is by text message to a personal mobile phone, but this will only be done if the participant has given permission. If texting is not possible or not permitted by the participant, the participant will be asked permission for other ways of contacting her should this be necessary. RU staff will only leave neutral messages that cannot be linked to RU, the WISH study, or the various diagnostic tests that were conducted (see below). When phone calls are made, RU staff will identify themselves with their first name only, and will then verify that they have the right person on the phone by asking them for their full name, date of birth, and the name of the study. Medical information will only be shared by phone with the participant herself. When phoning a contact person, RU staff will only provide their first name and a contact phone number. Participants will be informed of these contacting procedures during their clinic visit.

Our aim is to provide all study services within half a day (a morning or an afternoon). However, participants may elect to leave the clinic before all testing has been completed. This may be particularly relevant for women who are selected (by risk scoring tool) for CT/NG testing because the GeneXpert testing procedure takes about 90 minutes. In that case, participants will be contacted in the agreed-upon manner to let them know whether all results were negative (no need for a clinic visit) or whether they need to return to the clinic for further follow-up and/or treatment. We will use the following text messages: 'This is [first name of RU staff member]; all clear' when all results are negative, and 'This is [first name of RU staff member]; please visit us as soon as possible' when a clinic visit is needed. If a participant needs to return to the clinic but misses her visit, study staff will attempt to contact her again until the required follow-up has been conducted or three contact attempts have been made. The number of contact attempts will be recorded in the participant's records.

Participants may voluntarily withdraw from the study for any reason at any time. The Principal Investigator may also discontinue participants (for example, if this is deemed in the best interest of the participant). The reason(s) for withdrawal or discontinuation will be recorded in the participant's records.

6. STUDY PROCEDURES - CLINICAL

Curable urogenital infections (syphilis, NG, CT, TV, BV, vaginal candidiasis and UTI) will be treated at RU, and partner notification and treatment services will be offered if applicable, free of charge (see Appendix 14.3 for details). Participants will receive a full course of treatment in accordance with current Rwandan national treatment guidelines and, in the case of syphilis, NG, CT and TV, will be encouraged to bring regular sexual partners for treatment of the same infection. Treatment failure for all of these infections except for NG is rare if adherence is good, and standard of care for these infections does not include tests of cure (16, 17). Women will be advised that, if symptoms return, this is most likely due to reinfection by an infected sexual partner. They will be referred for treatment of this new infection to their local public clinic. Treatment failure for NG is expected to be more common due to rapid emergence of antibiotic resistance in this organism. Women who test positive for NG will therefore be actively followed-up by RU Physicians and will be offered a second-line treatment if the first line treatment fails.

Women will also receive reproductive health counselling and condoms free of charge. Any other medical conditions (including newly diagnosed HIV and pregnancy) and reproductive health needs (including contraception) will be actively referred to local clinics or referral hospitals. The RU study team is well connected with available services in Kigali.

The order of procedures at study visits as described below may be modified somewhat to improve clinic flow but informed consent procedures will always be done before any other procedures, and face-to-face interviewing will be done before counselling in order not to bias the interview results.

6.1 Schedule of assessments

See Appendix 14.1.

6.2 Procedures first WISH study visit (Main Visit)

Upon arriving at the RU research clinic all potentially eligible participants will be assigned a participant identification number after which the informed consent process will be done according to existing RU SOPs (see section 10.5 for details).

After written informed consent has been obtained, the following procedures will take place:

1. Collect contact information (see section 5.4 for details);
2. Complete eligibility checklist. Women who are menstruating at the time of this visit will be able to participate in most cases but may be asked to return after they have finished menstruating.
3. Complete face-to-face interview (sociodemographic characteristics, sexual behaviour, medical and reproductive history, and current urogenital symptoms) including the risk scoring questionnaire;
4. HIV/STI/BV risk reduction counselling;
5. Determine which additional services will be offered and ask the participant whether she wants to opt out of any services:
 - HIV, BV and TV testing: offered to all women
 - Pregnancy testing: offered to all women if indicated
 - UTI testing: offered only if UTI symptoms are reported
 - Syphilis, NG and CT testing: offered only if selected by risk scoring questionnaire
 - Management of vaginal candidiasis, lower abdominal pain, genital ulcers/inguinal bubos, and genital warts if relevant symptoms are reported
6. If applicable: Collect blood (one tube of about 10 ml; for HIV and syphilis testing), urine (for

pregnancy and urinalysis dipstick testing), and vaginal swabs (up to 5 swabs for BV, TV, and CT/NG testing, and temporary storage for research testing).

- Women can choose to have the vaginal swabs collected by self-sampling instead of clinician-sampling; vaginal swabs will be collected without the insertion of a speculum unless the clinician deems a speculum examination clinically necessary (see 10). Women who refuse collection of vaginal swabs altogether, and are selected for CT/NG testing, may have their urine tested instead (based on previous studies, we expect refusal of vaginal swab collection to be rare).
 - Women who refuse venepuncture will be offered HIV and syphilis testing by finger prick. However, they will be told that venepuncture will be necessary if the HIV or syphilis results come back positive for confirmatory testing.
 - Left-over blood, urine and two vaginal swabs will be temporarily stored for research testing (see 13 and sections 4.1 and 8.2).
7. If applicable: HIV, pregnancy, urinalysis dipstick, vaginal pH, TV POC test, and/or syphilis POC test in the onsite RU laboratory;
 8. If applicable: GeneXpert CT/NG test in the onsite RU laboratory. Women who are selected for this test should be told that the test will take approximately 90 minutes. In the meantime, continue with 9.
 9. Provision of POCT results, and provision of treatment and referrals as needed (in accordance with the latest version of the RU SOP for Reproductive Tract Infection Management and the RU SOP for Referrals; see also Appendix 14.3). At this point, also provide treatment for symptomatic vaginal candidiasis, lower abdominal pain, genital ulcers/inguinal bubos, and genital warts as required.
 10. Pelvic examinations will only be conducted when deemed necessary by the study physician (e.g. in women reporting lower abdominal pain).
 11. If necessary: schedule a follow-up text message, phone call, or visit (e.g. for provision of GeneXpert CT/NG results if the participant does not want to wait).
 12. Participant leaves the RU clinic.
 13. Further research testing after the participant has left the clinic to assess POCT performance:
 - Vaginal swabs: GeneXpert CT/NG test on all women who were not selected for testing by risk scoring questionnaire (so that a GeneXpert CT/NG test result is available for everyone)
 - Urine: The GeneXpert CT/NG test may be done on urine instead of vaginal swabs when vaginal swabs are not available.
- If funding permits:
- Vaginal swabs: Molecular testing for NG, CT, TV, and BV
 - Urine: Molecular testing for UTI.

6.3 Procedures any additional WISH study visits (Additional Visits)

Study staff may ask participants to return to the RU clinic if they require additional provision of test results, partner notification, treatment and/or referral. Other unscheduled visits may take place at the request of participants. At unscheduled visits, only the procedures for which the visit was intended will take place, and all of these will be documented.

7. STUDY PROCEDURES – QUALITATIVE RESEARCH

Two workshops will be held with study staff and investigators, reproductive health care clinicians, programme implementers and policymakers in Kigali, Rwanda. One will be held before the start of the study (its main purpose is to discuss the study and to decide on M&E indicators to measure during the study) and one after completion of the study (to discuss the study results, implications for urogenital infection care in Rwanda, and the potential roll-out of novel vaginal microbicides and

multipurpose prevention technologies for HIV and pregnancy prevention as soon as efficacious products become available). The workshops will not be taped, but study staff will take detailed notes of the discussions. These notes will form one source of data to address the qualitative research objectives. For data management/analysis and confidentiality, see sections 9 and 10.4, respectively.

In addition, a total of about 10 IDIs will be conducted with individual clinicians, programme implementers and policymakers to address the qualitative research objectives. The interviewees will be selected from among RU's professional network in Kigali, and an attempt will be made to include professionals with different types of relevant expertise. They will be approached by the Principal Investigator. Selected interviewees will be asked to provide written informed consent for the IDI. The IDI procedures will take place in a private location chosen by the interviewee. The interviews will be taped. For data management/analysis and confidentiality, see sections 9 and 10.4, respectively.

8. STUDY PROCEDURES - LABORATORY

8.1 Diagnostic tests

The following diagnostic tests will be performed in the onsite RU laboratory using validated test kits and SOPs:

- HIV by rapid test algorithm based on the most recent Rwandan HIV VCT guidelines, all tests locally available;
- Urine hCG pregnancy test, locally available;
- UTI by urinalysis dipstick test, locally available;
- TV by OSOM POC test (Sekisui Diagnostics, San Diego, CA, USA), to be imported;
- BV by EcoCare Vaginal pH Comfort Swab (Merete Medical, Luckenwalde, Germany), to be imported;
- Syphilis by Alere Determine POC test (Alere International, Galway, Ireland), regionally available;
- If syphilis treponemal POC test is positive, RPR (locally available) will be done to confirm current infection.
- GeneXpert CT/NG test (Cepheid, Sunnyvale, CA, USA), to be imported.

All RU staff handling specimens have been vaccinated against Hepatitis B. The RU SOPs for Universal Precautions, Accidental Exposure to Blood and Body Fluids, Disposal of Hazardous Waste and Medical Emergencies will be followed.

8.2 Research tests

Research tests include:

- Stored swabs from all women who did not undergo GeneXpert CT/NG POC testing at their Main Visit will be tested by GeneXpert before the end of the funding period (and as soon as possible after their Main Visit) so that we have a GeneXpert result for all women. These tests will be conducted in the onsite RU laboratory.
- If funding permits, we will also test stored swabs from all women by molecular tests for TV, BV and UTI. These tests will be conducted at UoL within two years after termination of data collection in Rwanda.

These research tests will allow for sensitivity, specificity, PPV, NPV and cost effectiveness comparisons of syndromic management, POC testing after risk scoring, and POC testing of all women, and will improve our understanding of organisms associated with BV and UTI.

Specimens will be processed and temporarily stored (at -80°C, or -20°C if -80°C is not possible) at RU and either tested by GeneXpert in the onsite laboratory at RU or shipped in batches from RU to UoL

using RU SOPs (which are based on local and international shipping regulations) and Material Transfer Agreements. Specimens that are shipped to Liverpool will be labelled with ID numbers and specimen collection dates, but not with names or other personal identifiers. The electronic participant identification register (ePIR; see section 9), which is the only database that will link ID numbers with names and contact information, will remain at RU in Rwanda and the study team in Liverpool will not have access to it. After arrival at UoL, specimens will be stored until testing at -80°C in the Ronald Ross building, Institute of Infection and Global Health (IIGH), under the custodianship of Prof. Janneke van de Wijgert. Sample storage and testing at UoL will be in accordance with the Human Tissue Authority Code of Practice 9 (see www.hta.gov.uk/guidance/codes_of_practice.cfm), UoL Code of Practice for the Control of Substances Hazardous to Health (COSHH), and IIGH SOPs. IIGH is used to handling specimens containing micro-organisms (dead or alive), including specimens containing HIV. The specimens collected and stored in this study will not contain viable HIV but may contain dead virus. Prior to commencement of any laboratory procedures, Prof. Janneke van de Wijgert will prepare the required risk assessments and will reach agreement on the specific procedures to be used with the Departmental Safety Advisor and University Safety Advisor. All UoL staff handling study specimens have been vaccinated against Hepatitis B.

9. DATA MANAGEMENT AND ANALYSIS

We will use two separate custom-built databases with automated quality control checks. The ePIR will contain names, identification numbers, contact information, and clinic visit attendance; the information will be entered directly into the database by a receptionist when the client first enters the clinic. The ePIR will be kept at RU on a stand-alone password-protected desktop computer with access limited to key project staff, and is backed up on a separate stand-alone password-protected hard drive (under the custodianship of Dr. Stephen Agaba) once per week. The second database will contain clinical data (such as sociodemographics, risk score, current genitourinary symptoms, services offered and accepted/declined, laboratory test results, and treatments/referrals given) by participant identification number and visit date, but will not contain any personal identifiers. The clinical data will be entered on paper-based medical records and laboratory forms when the participant is in the clinic, and will be entered into the database when all procedures for that participant have been completed. This database will be programmed by a professional database programmer (Robert Meester) using OpenClinica software, with password-protection at different access levels for different key staff, and with automated daily back-ups. This database and back-ups will be kept on a remote secure server (under the custodianship of Robert Meester). All paper-based forms will be kept at RU in lockable cabinets and/or rooms for confidentiality reasons. Documents containing names and/or signatures (such as consent forms) will be kept separately from all other documents containing identification numbers only.

Note-takers will take notes in English during the workshops. The IDIs will be tape-recorded, transcribed verbatim, and translated into English (if the interview was not conducted in English). The workshop notes and English translations of IDI transcripts will be analysed using Nvivo software. The tapes will be destroyed after all data have been processed, information in documents that might identify interviewees will be anonymised, and reports will contain aggregate data only. The anonymised transcripts, Nvivo data, and workshop reports will be kept on a password-protected RU desktop computer with access limited to key project staff and regular back-up on a separate stand-alone password-protected hard drive (under the custodianship of Dr. Stephen Agaba).

Use of the data, and especially publication in the peer-reviewed literature, will be governed by a project-specific 'data-sharing and publication policy'. For archiving procedures, see section 12.7.

10. ETHICAL ISSUES AND PROCEDURES

10.1 Ethical review

The protocol will be reviewed by the Chief Investigator, Principal Investigator, and co-investigators, and approved by the Chief Investigator and Principal Investigator. Prior to enrolment of the first participant, the protocol will be approved by the Rwanda National Health Research Committee (this is an administrative approval, not an ethics approval), and two ethics committees: the National Ethics Committee of Rwanda and the UoL Research Ethics Sub-committee for Physical Interventions.

10.2 Burdens and benefits

Physical burdens may include discomfort and bruising at the site of venepuncture and discomfort in the vagina during collection of vaginal swabs or pelvic examinations. Psychological burdens may include embarrassment during the face-to-face interview and sample collection procedures, and personal and/or interpersonal stress if HIV infection and/or an STI is diagnosed. Though unlikely, a breach of confidentiality is a potential risk. Furthermore, women may have to spend a significant amount of time at the study clinic (2-4 hours).

Benefits for all participants include free HIV counselling and testing, free pregnancy testing, and free diagnosis and treatment of several common STIs, BV, vaginal candidiasis, and UTIs. In addition to treatment and referral for themselves, participants will be offered partner notification services if they are newly diagnosed with HIV or another STI. They will also receive male condoms free of charge.

Workshop and IDI participants will not be exposed to any specific burdens or benefits, except for the time spent on these activities. They might contribute to improving urogenital health care in Rwanda.

10.3 Compensation and insurance

Participants will not receive any monetary compensation or reimbursements. They will be offered refreshments while waiting to be seen by a study clinician, or while attending the workshops or IDIs.

UoL will obtain malpractice/no-fault liability insurance covering the entire conduct of the study. This insurance will cover the trial participants and study staff for any damage or injury that results from any study-related activities or procedures.

10.4 Confidentiality

Every attempt will be made to maintain the confidentiality of study participants. All women will be assigned a unique personal identification number for use on all study forms containing interview, clinical, and laboratory data. This identification number will be linked to women's personal information in a central registry (the ePIR). Documents containing the names and/or signatures of participants (such as consent forms) will be kept separately from all other study documents containing identification numbers only. Each specimen will also be given a unique identification number to link them to other laboratory results and questionnaire data of the same person at the same visit. In IDIs, participants will be told they can use a name other than their given name for confidentiality. Transcripts from the IDIs will not contain participants' given names. The workshop reports will contain a list of workshop attendees, but will not specify which opinions were voiced by which individuals.

All interviews, counselling procedures and clinical procedures will be conducted in private and study staff will be told not to share confidential information regarding study participants with anyone outside the study team without a participant's consent. All completed study forms and documents will be kept in lockable rooms and/or cabinets at RU. Care will be taken to maintain confidentiality during follow-up. Study results will be presented as aggregated data, with no personal information.

10.5 Informed consent procedures

Written informed consent will be obtained from all study participants. The informed consent form (ICF) has to be co-signed by a parent or guardian when the participant is aged 18-20 and unmarried (by Rwandan law, women aged 18-20 who are legally married are no longer considered legal minors and can sign for themselves). Participants with insufficient literacy can provide a thumbprint but the informed consent process should be observed by an independent witness who co-signs the ICF. The witness cannot be a RU staff member, but can be another study participant.

Two separate participant information sheets (PIS) and ICFs will be used: one for the cross-sectional study and one for the IDIs. In the cross-sectional study, the informed consent process may start with a video or group session in the waiting room, but will always be followed by an individual session attended by the participant and, if applicable, her parent/guardian and/or her witness. The consent procedures will be conducted in the language chosen by the participant and will be completed before study procedures take place. In all cases, two copies of the PIS and ICF documents will be provided to the participant (and her parent/guardian and/or witness if applicable); they will be asked to leave one fully signed copy at RU for the study files and to take one fully signed copy home for their own records.

Participants will be asked to sign separately for long-term storage of specimens for future testing that is not currently specified in this study protocol. Any additional testing would be formally approved by the study sponsor and the ethics committees listed in section 10.1 via a protocol amendment prior to initiation of testing. If a participant does not consent to long-term storage, her specimens will be destroyed after the primary and secondary study objectives have been achieved. Similarly, participants will be asked to sign separately for their anonymised data to be used in future research studies by third parties in line with open data requirements. If a participant does not consent to this, her anonymised data will not be released to third parties.

Participants can take the PIS and ICF home to think it over or discuss with others and come back on another day. After having signed the consent form, participants are free to withdraw at any time. This would entail notifying any RU staff member of the withdrawal. Data and samples that were already collected up to that point would be discarded.

11. INVESTIGATIONAL PRODUCTS

In this study, no investigational products will be used.

12. OTHER

12.1 Funding

This study is funded by the European and Developing Countries Clinical Trials Partnership (EDCTP). The main funding contract is between EDCTP and the UoL IGH (grant number EDCTP-CSA MI -2014-592) and by subcontract between UoL IGH and RU and UoL IGH and ITM.

12.2 Study management and oversight

The trial sponsor is UoL and this protocol and other study documentation was therefore reviewed and approved by the UoL Sponsorship Committee. Overall study management at UoL will be coordinated by the Chief Investigator Prof. van de Wijgert in the Institute of Infection and Global Health. She will make use of resources available through the UoL Research Support Office.

Study implementation in Rwanda will be managed by the RU team, led by the Principal Investigator Dr. Stephen Agaba. He will work closely with the RU community outreach, clinical, laboratory, data management, and social science team leaders. All clinical and qualitative research procedures (with the possible exception of the IDI's with stakeholders), as well as diagnostic laboratory testing, will take place at RU according to RU SOPs and using RU source documents.

12.3 Protocol amendments

This study will be conducted in full compliance with the most recent approved version of the study protocol. Protocol amendments will be reviewed and approved by the investigators and ethical review committees prior to implementing the amendment, except when necessary to protect the safety, rights and wellbeing of study participants.

12.4 Good clinical practice

This study will be implemented in accordance with the ICH-Good Clinical Practice (GCP) and Good Clinical Laboratory Practice (GCLP) guidelines. We will implement the following monitoring procedures:

- The Chief Investigator will travel to Kigali to conduct the study training and study initiation visit.
- During implementation of the demonstration project, daily internal quality control procedures will be implemented in accordance with the RU SOP for Data Management and Quality Control (which is based on ICH-GC(L)P).
- A UoL project manager will conduct two monitoring visits during implementation of the demonstration project.
- The Chief Investigator will travel to Kigali again to conduct the study close-out visit.

12.5 Study termination

If the study is prematurely terminated or suspended, the Chief Investigator will promptly inform the investigators/institutions involved in the study, the ethics committees listed in section 10.1, the Rwanda National Research Committee, and the Rwanda Ministry of Health of the termination or suspension and the reason(s) for the termination or suspension. The RU Principal Investigator is responsible for informing active study participants of study termination or suspension.

12.6 Dissemination of results

Findings from this project will be disseminated at a workshop in Kigali after the completion of the study as described in section 7. They will also be presented at national and international conferences, and published in peer-reviewed journals. Findings will be reported back to the study participants and their communities by RU staff with the help of the community mobilisers.

12.7 Archiving

Study records and data will be kept at RU according to the RU SOP for Archiving. RU will retain all

study records and data until all data analyses and report-writing have been completed, after which they will be destroyed. Study records and data may not be destroyed without prior written approval from the Chief Investigator.

After database lock, all anonymised study data will also be kept on the UoL M-drive (under the custodianship of the Chief Investigator) until all data analyses and report-writing has been completed. After that, the anonymised study data will be archived in the UoL RDM DataStore (<https://www.liverpool.ac.uk/csd/research-data-management/>).

All data generated in this project will be co-owned by RU and UoL. However, after database lock, the anonymised data will be shared with project partner ITM Antwerp and with the Ministry of Health in Rwanda. The anonymised data may also be made available to other interested parties using RDM DataStore facilities on a case-by-case basis. The ePIR will never be shared with third parties to protect participant confidentiality.

13. REFERENCES

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14. APPENDICES

14.1 Schedule of assessments

Procedures	Main visit	Additional visits	IDI
Informed consent	X		X
Assign PIN and contact information	X		X
Eligibility checklist	X		
Face-to-face interview incl. risk scoring/symptoms, quantitative	X		
Face-to-face interview, qualitative			X
Offer services and ask women if they want to opt out of any services	X	X ¹	
HIV/STI/BV risk reduction counselling	X	[X]	
If not opted out: Venepuncture (10 ml) or finger prick	X		
- Rapid HIV testing ²	X		
- If pregnant or selected by risk scoring: Syphilis POCT	X		
If not opted out and indicated: Urine collection	X		
- If indicated: hCG urine pregnancy testing	X		
- If UTI symptoms: Urinalysis dipstick testing	X		
If not opted out: Collection of vaginal swabs without speculum (choose if self-sampling or clinician-sampling)	X		
- Vaginal pH and TV POCT	X		
- If selected by risk scoring: CT/NG POCT	X		
Gynaecological exam ³	[X]	[X]	
Provide POCT results	X	X	
Provide treatment based on POCT results and management of genital ulcers/inguinal bubos, vaginal candidiasis and lower abdominal pain if relevant symptoms reported	[X]	[X]	
Provide partner notification and treatment		[X]	
Provide referrals	[X]	[X]	
Client satisfaction survey (on a subsample)	X		
Laboratory procedures at end of study			
Vaginal swabs:			
- CT/NG POCT on stored samples from women who were not tested before	X		
- If funding permits: NG, CT, TV, BV PCRs on all stored samples	X		
Left-over urine:			
- If funding permits: molecular testing of stored samples to determine organisms associated with UTIs	X		

BV=bacterial vaginosis; CT=*Chlamydia trachomatis*; FU=follow-up; hCG=human chorionic gonadotropin; IDI=in depth interview; NG=*Neisseria gonorrhoeae*; PIN=participant identification number; PCR=polymerase chain reaction; POCT=point-of-care test; STI=sexually transmitted infection; UTI=urinary tract infection; TV=*Trichomonas vaginalis*. [x] = only if indicated.

1. Only procedures for which the visit was intended will be carried out.
2. By the algorithm recommended in the most recent Rwandan HIV voluntary counselling and testing guidelines.
3. Gynaecological exams will only be done when deemed necessary by the study physician (for example, in the case of genital ulcers or lower abdominal pain).

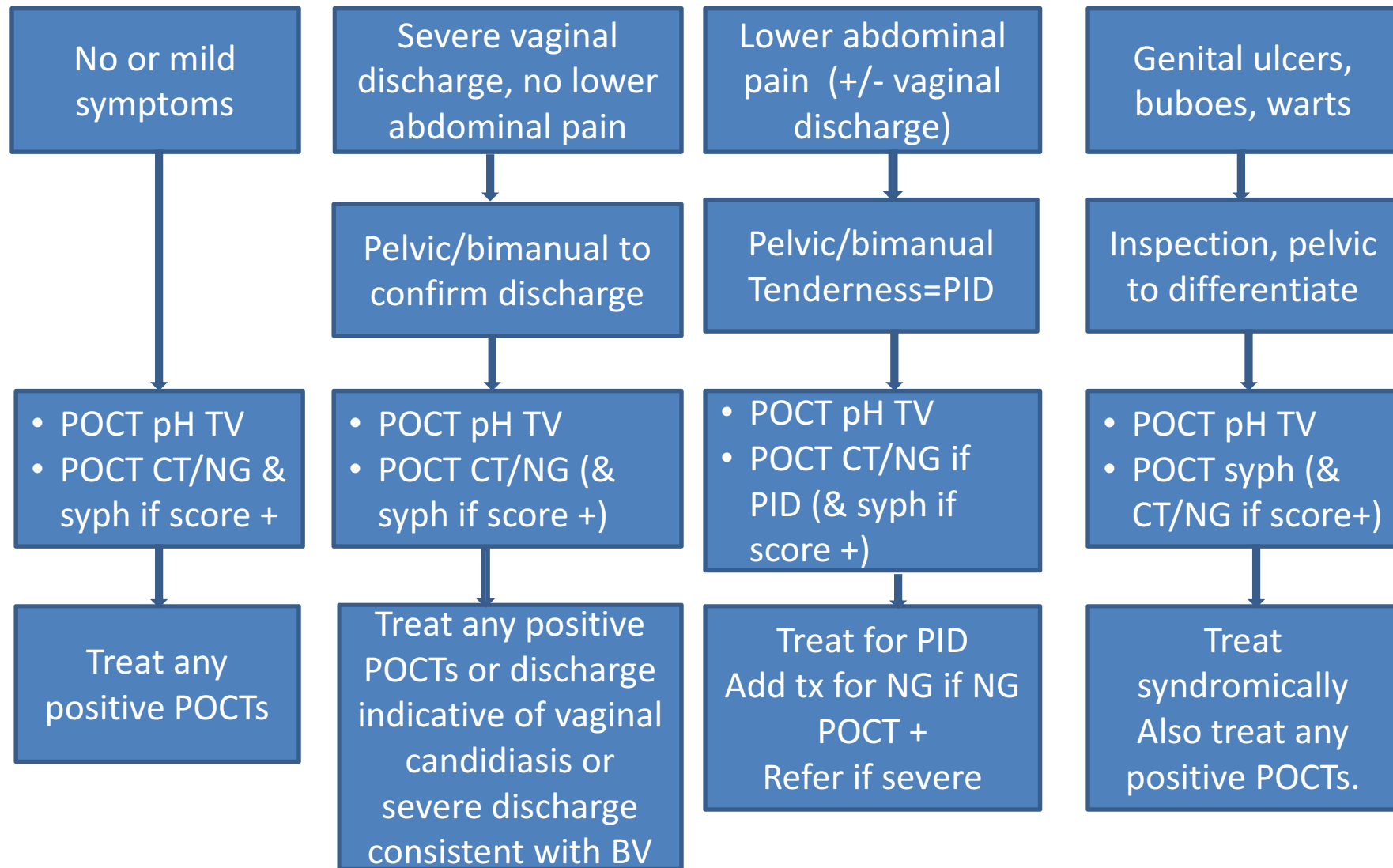
14.2 WISH clinical algorithms

See attached as a separate document

14.3 WISH treatment guidelines by etiologic or syndromic diagnosis

See attached as a separate document

Appendix 14.2: WISH clinical algorithms



CT= *Chlamydia trachomatis*; NG= *Neisseria gonorrhoeae*; pH=vaginal pH; PID=pelvic inflammatory disease; POCT=point of care test; syph=syphilis; TV= *Trichomonas vaginalis*.

APPENDIX 14.3: WISH TREATMENT GUIDELINES BY ETIOLOGIC OR SYNDROMIC DIAGNOSIS

Diagnosis	Treatment (follow latest Rwandan guidelines)	Other Follow-Up
Gonorrhea Positive GeneXpert NG test. Treat regardless of the presence of symptoms.	1 st choice: Ciprofloxacin 1g single oral dose 2 nd choice: Ceftriaxone 250 mg in one single IM dose	<ul style="list-style-type: none"> - In female sex workers, also consider the possibility of rectal or pharyngeal NG (take throat and rectal swabs for testing if suspected). - Actively follow-up participants with laboratory-confirmed NG approximately 7 days after treatment to check for treatment failure (due to NG resistance). If symptoms are still reported, give the 2nd choice treatment. - Male partners should be treated in the same manner as the female index case. Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Chlamydia Positive GeneXpert CT test. Treat regardless of the presence of symptoms.	1 st choice: Doxycycline 100 mg twice per day for 7 days 2 nd choice: Erythromycin 1g twice per day for 7 days	<ul style="list-style-type: none"> - Most CT infections are asymptomatic so active follow-up after treatment is not useful. - Male partners should be treated in the same manner as the female index case. Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Trichomoniasis Positive TV OSOM test. Treat regardless of the presence of symptoms.	1 st choice: Metronidazole 2 g single oral dose OR Metronidazole 400 or 500 mg orally twice per day for 7 days 2 nd choice: Tinidazole 2g single oral dose OR Tinidazole 500 mg twice per day for 7 days	<ul style="list-style-type: none"> - If BV is also present (see below), the 7 day treatment is preferred because it is more effective against BV. However, if poor adherence is suspected, use a single dose treatment. - Male partners should also be treated in the same manner as the female index case. Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Bacterial vaginosis Vaginal pH is 5.0 or higher. Treat regardless of the presence of symptoms.	1 st choice: Metronidazole 400 or 500 mg orally twice per day for 7 days OR Metronidazole 2g single oral dose. 2 nd choice: Tinidazole 2g orally once a day for 2 days or 1g per day for 5 days. Alternative: Clindamycin 300 mg orally twice per day for 7 days	<ul style="list-style-type: none"> - Metronidazole 2g single oral dose is a less effective treatment for BV than the 7-day treatment. Only give a single oral dose if you are worried that the patient will not be adherent for 7 days. - Women should be encouraged to wear clean underwear daily, not wash inside the vagina (and if this cannot be avoided, to use clean plain water only), and use hormonal contraception to prevent recurrence. - Male partners are not treated but women should be told that BV-associated bacteria can be passed between sexual partners. Their male partners should be encouraged to improve their penile hygiene and clean underneath the foreskin regularly.
Vaginal candidiasis When symptoms typical for vaginal candidiasis are present: White, curd-like, odorless vaginal discharge with vaginal itching and/or burning.	1 st choice: Fluconazole 150 mg single oral dose 2 nd choice: Clotrimazole 200 mg pessaries every night for 3 nights	<ul style="list-style-type: none"> - Do <u>not</u> treat without lab confirmation; do <u>not</u> treat if asymptomatic) - Male partners are not notified but if a male partner has symptoms of balanoprostitis, he can be given 1% Clotrimazole cream to be applied for 14 days. - Patients should be counseled to abstain from sex or use condoms, until symptoms have cleared.

Diagnosis	Treatment	Other Follow-Up
Active primary syphilis When treponemal test (Determine) is positive and non-treponemal test (RPR) has been positive for < 2 years. Treat regardless of the presence of symptoms (symptoms associated with primary syphilis: Painless, rubbery, mobile genital sores).	1 st choice: Benzathine benzyl penicillin 2.4 million IU IM single dose. Alternatives: Erythromycin 1g orally 2x per day x 14 days OR Doxycyclin 100 mg orally twice per day x 14 days	<ul style="list-style-type: none"> - Male partners should also be treated. If the woman has primary syphilis, her male partner(s) may have past, primary, secondary or neurosyphilis. For this reason, the male partner(s) should be seen by a RU Physician to determine which syphilis treatment is required. Syphilis testing of the male partner may be conducted. However, even if his tests show past instead of active infection, treatment for primary syphilis should be given because of recent exposure to the index case. - Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Active secondary syphilis or active syphilis of unknown duration When treponemal test (Determine) is positive and non-treponemal test (RPR) has been positive for > 2 years or for unknown duration. Treat regardless of presence of symptoms (symptoms associated with secondary syphilis: generalised skin lesions, many others).	1 st choice: Benzathine benzyl penicillin 2.4 million IU IM once a week x 3 weeks Alternatives: Erythromycin 500 mg orally 3x per day x 30 days OR Doxycyclin 100 mg orally twice per day x 30 days	<ul style="list-style-type: none"> - Ask if the participant has ever been treated in the past. However, if the treponemal and non-treponemal tests are both still positive, treat anyway regardless of past treatments. - Male partners should also be treated. See active primary syphilis above. - Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Neurosyphilis When treponemal (Determine) and non-treponemal (RPR) tests are both positive and there is neuro-muscular involvement.	Refer to a hospital as a matter of urgency for intravenous Benzathine benzyl penicillin treatment.	<ul style="list-style-type: none"> - Male partners should also be treated. See active primary syphilis above. - Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Syndrome: Vaginal discharge without lower abdominal pain Conduct speculum and bimanual examination and treat only if cervical and/or vaginal discharge is seen and lower abdominal pain is ruled out.	<ul style="list-style-type: none"> - If mucopurulent discharge from the cervix or cervicitis are present: Treat for NG, CT, and TV as described above; - If mucopurulent discharge from the cervix or cervicitis are NOT present: Treat for BV, vaginal candidiasis, and TV as described above. 	<ul style="list-style-type: none"> - Partner notification and treatment is not done but women will be advised to encourage their male partner(s) to get tested themselves. To avoid social harms, we will explain to women that their vaginal discharge may be physiological and that we did not confirm the presence of an STI. - Women should be advised to abstain from sex or use condoms during treatment just in case they do have a STI.
Syndrome: Lower abdominal pain or deep dyspareunia with or without vaginal discharge (=PID) <ul style="list-style-type: none"> - RU Physician to conduct a speculum and bimanual examination; diagnose acute PID only in the case of cervical motion, uterine, or adnexal tenderness. - Conduct CT/NG testing. - Refer to the hospital in case of a missed period, recent delivery, abortion, or miscarriage, abdominal guarding or rebound tenderness, abnormal vaginal bleeding, or abdominal mass. Also refer to the hospital if the PID is severe (e.g. temp>38 °C). 	If PID confirmed by RU Physician: 1st choice: Ciprofloxacin 500mg twice per day for 3 days AND Doxycycline 100 mg twice a day for 14 days AND Metronidazole 500mg twice a day for 14 days. Add Ceftriaxone 250 mg IM single dose if the participant is NG-positive. Alternatives: Ceftriaxone 250 mg IM in a single dose AND Erythromycin 1g twice per day for 14 days with meals AND Tinidazole 1g per day for 14 days.	<ul style="list-style-type: none"> - Actively follow-up participants with laboratory-confirmed NG approximately 7 days after treatment completion to check if the PID symptoms have improved. If not, treatment with additional antibiotics should be considered to cover potentially - Male sexual partners will be tested for CT/NG, and will receive treatment according to those test results. If both tests are negative, they will not receive any treatment. - Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.

Diagnosis	Treatment	Other Follow-Up
Syndrome: Genital vesicles with or without inguinal buboes with the notion of recurrence (= genital herpes) - RU Physician to inspect and palpate external genitalia/inguinal area and conduct speculum exam to differentiate between the various genital ulcer/bubo syndromes and genital warts. Note: herpetic vesicles are usually painful.	If confirmed by RU Physician: 1st (and only) choice: Acyclovir 400 mg orally 3 times per day x 7 days and 5 days in case of recurrence	- In female sex workers, also consider the possibility of anal genital vesicles - The treatment will not cure genital herpes but will reduce HSV-2 shedding and onward transmission, and may reduce the duration of the outbreak. - Partner notification and treatment is not indicated but the women should be advised to abstain from sex or use condoms during outbreaks to HSV-2 transmission to sexual partner(s).
Syndrome: Genital ulcers not suggestive of genital herpes with or without inguinal buboes - RU Physician to inspect external genitalia/inguinal area and conduct speculum exam to differentiate between the various genital ulcer/bubo syndromes and genital warts.	If confirmed by RU Physician: Treat for syphilis (as described above) and chancroid: Ciprofloxacin 500 mg orally twice per day for 3 days (Alternative: Ceftriaxone 250 mg in one single IM dose)	- In female sex workers, also consider the possibility of anal genital ulcers - Male partners should receive the same treatments. - Couples should be treated around the same time and counseled to abstain from sex or use condoms during treatment to avoid re-infections.
Syndrome: Inguinal buboes without ulcers - RU Physician to inspect and palpate external genitalia/inguinal area and conduct speculum exam to differentiate between the various genital ulcer/bubo syndromes and genital warts.	If confirmed by RU Physician: Treat for chancroid (as described above) and LGV: Doxycycline 100mg twice per day for 21 days.	- In female sex workers, also consider the possibility of anal LGV. Anal LGV may not cause inguinal buboes but may cause proctocolitis symptoms instead. - Male partners should receive the same treatments. Couples should be treated around the same time and counseled to abstain from sex or use condoms to avoid re-infections.
Syndrome: Genital warts/condylomata - RU Physician to inspect and palpate external genitalia/inguinal area and conduct speculum exam to differentiate between the various genital ulcer/bubo syndromes and genital warts.	- In the case of mild warts, provide treatment with 20% podophyllin solution. - In the case of severe warts/condylomata, refer for cryotherapy.	- In female sex workers, also consider the possibility of anal genital warts - Treatment of male partners is not indicated. Women should be advised to abstain from sex or use condoms during outbreaks to avoid HPV transmission to sexual partner(s).
Urinary tract infection - Do urinalysis test only when UTI symptoms are present. - Treat when urinalysis test shows at least 1+ leukocytes or nitrites. Also consider treatment if there is blood in urine that cannot be explained by menstrual blood or spotting related to contraceptive use.	- 1 st choice: Ciprofloxacin 500 mg orally twice per day x 7 day. Alternative: Ceftriaxone IM 125 twice per day x 5 days.	- No follow-up and no male partner treatment required.
Cervical cytology (not done at RU)	- Refer all high-risk women for cervical cancer screening if they have not undergone screening in the past five years.	- No male partner referral required.

CONTRAINDICATIONS:

- Doxycycline, tetracycline, ciprofloxacin and cotrimoxazole should not be used during pregnancy and lactation; doxycycline may reduce the effectiveness of hormonal contraception. Azithromycin and ceftriaxone may be used by pregnant women.
- Metronidazole and fluconazole can be used only once during the first trimester of pregnancy; patients must not drink alcohol while taking metronidazole.
- Benzathine benzyl penicillin is contraindicated in case of allergy or when using hormonal contraception.
- Doxycycline and erythromycin should be taken with meals.