STUDY PROTOCOL

Preparing for a clinical trial of interventions to maintain normal vaginal microbiota for preventing adverse reproductive health outcomes in Africa

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Protocol Approval:

Chief Investigator
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Signature
Date: 24 April 2015

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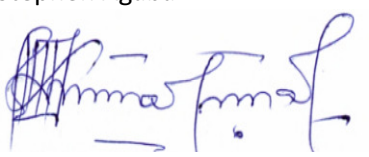


Signature
Date: 24 April 2015

STATEMENT OF PROTOCOL COMPLIANCE AND CONFIDENTIALITY

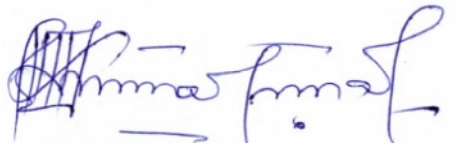
By signing this statement, the Principal Investigator agrees to:

- 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 and in full agreement with all applicable regulations and the international guidelines regarding the conduct of clinical research.
- Permit trial-related monitoring, audits, independent ethics committee review, and regulatory inspections, providing direct access to source data/documents during and after the course of the trial.
- 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 (ICH) Good Clinical Practice (GCP) (1) 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 (UoL), 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 (RU), 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



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Signature | Date

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LIST OF ACRONYMS

AE	Adverse event
BV	Bacterial vaginosis
CGR	Centre for Genomics Research
(e)CRF	(electronic) Case report form
CVL	Cervicovaginal lavage
CT	*Chlamydia trachomatis*
EC	Executive committee
FDA	Food and Drug Administration
FGD	Focus group discussion
GBP	Great Britain pound
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GMP	Good Manufacturing Practice
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
MRC	Medical Research Council
NG	*Neisseria gonorrhoea*
PCR	Polymerase chain reaction
PIS	Participant information sheet
PPM	Potentially pathogenic micro-organism
qPCR	Quantitative polymerase chain reaction
RU	Rinda Ubuzima
RWF	Rwandese franc
SAE	Serious adverse event
SOP	Standard operating procedure
(e)SPIR	(electronic) Study participant identification register
STI	Sexually transmitted infection
SUSAR	Suspected unexpected serious adverse event
TSC	Trial steering committee
TV	*Trichomonas vaginalis*
UoL	University of Liverpool
UK	United Kingdom
USA	United States of America
UTI	Urinary tract infection
VMB	Vaginal microbiome
WHO	World Health Organisation

1. PROTOCOL TEAM ROSTER

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2. SYNOPSIS

Purpose	Women who are treated with oral antibiotics for a vaginal infection (such as bacterial vaginosis or BV) are left with a vulnerable vaginal microbiome (VMB). This often leads to recurrent BV. The purpose of this study is to determine the feasibility, and collect pilot data, on interventions to restore and maintain a healthy VMB for 2-6 months after oral antibiotic treatment for vaginal infection. This is important because BV is associated with increased HIV acquisition, adverse pregnancy outcomes, and maternal/neonatal infections. The data will be used to choose the best interventions and to inform the design of a future efficacy trial.
Study Design	Sixty HIV-negative, non-pregnant, sexually active women aged 18-45 with BV by Amsel criteria and/or *Trichomonas vaginalis* (TV) on wet mount or by culture will be treated using oral metronidazole for 7 days. After successful treatment (defined as no BV by Amsel criteria and no TV on wet mount) and when they are free of vaginal candidiasis, other curable sexually transmitted infections (STIs) and urinary tract infection (UTI)), they will be randomised to 4 different VMB maintenance interventions (15 per group) within 3 days of completing oral metronidazole treatment: 1) Behavioural 'vaginal practices cessation and safer sex' counselling only (control); 2) Behavioural counselling plus 0.75% metronidazole vaginal gel, one applicator (5g) twice weekly for two months; 3) Behavioural counselling plus Ecologic Femi vaginal capsule, once per day for 5 days immediately after oral metronidazole treatment followed by thrice weekly, for two months; 4) Group 4: Behavioural counselling plus Gynophilus vaginal tablet, once every 4 days for two months. In all 3 biomedical intervention groups, vaginal product use may be ceased temporarily during menstruation. Participants will be asked to adhere to the interventions for 2 months, and VMB assessments will take place before (screening and enrolment visits), during (Day 7, Month 1 and Month 2 visits), and after the interventions (Month 6 visit). Any episode of symptomatic BV by Amsel criteria, symptomatic vaginal candidiasis, STI or UTI diagnosed during follow-up will be treated using standard oral therapies and the interventions will be allowed to continue during this treatment. A subgroup of 3 women in each study group (N=12) will be asked to self-collect vaginal swabs at home every Monday, Wednesday, and Friday for the first month of the intervention to allow for an in-depth evaluation of VMB adjustments to the initiation of the interventions (the 'frequent sampling subgroup').
Study Site	Rinda Ubuzima (RU), Kigali, Rwanda
Study Products	• Vaginal antibiotic: 0.75% metronidazole vaginal gel (Graceway Pharmaceuticals, Bristol, TN, USA) • Vaginal probiotic: Ecologic Femi vaginal capsule (Winclove Probiotics, Amsterdam, The Netherlands) • Vaginal probiotic: Gynophilus vaginal tablet (Probionov, Clermont-Ferrand, France)
Study Population	HIV-negative, non-pregnant, sexually active women between 18-45 years at high risk of HIV, STIs, and BV in Kigali, Rwanda. RU has worked with this target population for 10 years. The BV prevalence was at least 40% in previous studies.
Study Duration	One year
Study Endpoints	Primary clinical endpoints will be the safety and preliminary efficacy of the study products, the latter defined as incidence of BV by Amsel criteria, BV by

	Nugent score (Nugent 7-10), intermediate microbiota (Nugent 4-6), TV (by culture) and symptomatic vaginal candidiasis (by wet mount) during follow-up. Secondary clinical endpoints will be membership of specific VMB clusters over time as determined by clustering analysis of all available Illumina MiSeq data, the presence over time of individual VMB bacteria (as determined by Illumina MiSeq), and the incidence of STIs (HIV, syphilis, herpes simplex type 2 (HSV-2), *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG)), and UTI (by urinalysis dipstick) during the follow-up period. In addition, we will conduct focus group discussions (FGDs), in-depth interviews (IDIs) and a quantitative survey to address specific feasibility objectives.

3. INTRODUCTION

3.1 Background

Microbiota are bacterial communities (consisting predominantly of commensal bacteria) that live in or on the human body, including the vagina. Under normal circumstances, these bacterial communities do not cause disease, and humans are dependent on them for protection against pathogens and for specific bodily functions (such as the processing of food in the gut). Healthy vaginal microbiota (VMB) consist predominantly of lactobacilli, which are thought to restrict growth of other bacteria by keeping the vagina acidic and via other antimicrobial mechanisms (2). Clinical conditions associated with an imbalanced VMB include bacterial vaginosis (BV) and vaginitis. In these conditions, lactobacilli are replaced by other micro-organisms that can cause symptoms, such as a high diversity of anaerobic bacteria in BV and potentially pathogenic micro-organisms (PPMs, such as streptococci, staphylococci, and *Escherichia coli*) in vaginitis. Like commensals, PPMs are harmless under normal circumstances. However, they are more likely to cause severe invasive disease under special circumstances such as wound infections and sepsis in vulnerable patients (3). In pregnant women, Group B streptococci are most feared as a frequent cause of maternal and neonatal infections and mortality (4).

BV is a complex condition involving multiple bacteria and can therefore be defined in multiple ways. In clinic settings, it is often diagnosed based on patient-reported symptoms alone or using the Amsel criteria (5). In the case of Amsel criteria, BV is diagnosed when 3 of the following 4 criteria are present: 1) clue cells on wet mount microscopy (a wet mount is vaginal fluid on a microscopy slide); 2) a 'fishy' odour after adding 10% KOH to the wet mount; 3) vaginal pH > 4.5; and 4) thin, homogenous vaginal discharge present. Both patient-reported symptoms as well as the Amsel criteria have poor sensitivity because many women with BV are asymptomatic. Therefore, in research settings, Gram stain Nugent scoring is more often used (6). Vaginal fluid is smeared on a microscopy slide, stained, and scored under the microscope based on the presence and relative quantity of three bacterial morphotypes (shapes). In this case, the diagnosis does not depend on the presence of signs or symptoms and is therefore more sensitive. A Nugent score of 0-3 is considered normal, 4-6 is referred to as intermediate microbiota, and 7-10 BV. Unfortunately, Nugent scoring is labour-intensive and is therefore usually not a feasible alternative to the Amsel criteria. Other micro-organisms that are often found in the vagina include *Candida* yeasts, *Trichomonas vaginalis* (TV) and other sexually transmitted pathogens. Vaginal candidiasis and TV are typically diagnosed by visualising the organisms on the wet mount by microscopy and/or by culturing them, and other sexually transmitted pathogens by commercialised pathogen-specific diagnostic assays.

Epidemiological studies using the Amsel criteria or Nugent scoring have shown that BV increases risk of preterm birth and infections in pregnant women and their neonates (7). Furthermore, BV and intermediate microbiota by Nugent score, TV and vaginal candidiasis, have been associated with increased transmission of HIV and other chronic sexually transmitted infections (STIs) (8). Studies have also shown that the female urinary tract has its own microbiome that resembles the VMB, and presence of *E. coli* in the VMB might therefore increase risk of urinary tract infections (UTIs) (9).

In the last decade, phylogenetic analyses of vaginal samples have shown that the VMB is more complex than previously thought (10-14). In these studies, vaginal samples were taken, and the bacterial DNA in those samples was characterised using molecular methods. The molecular method that is currently the most commonly used is sequencing of a portion of the bacterial 16S ribosomal RNA gene; this gene is ubiquitously present in bacteria (not in human cells) and can be used to identify bacteria at the genus or even species level because of differences in the nucleotide sequences of the gene. Using such molecular methods, several bacteria were identified that had

previously been missed because they were difficult to culture (10), and important differences were discovered between different species of the genus *Lactobacillus*. In a landmark study in women in the U.S. without vaginal symptoms, 5 VMB clusters were identified, 4 of them dominated by *L. crispatus*, *L. iners*, *L. gasseri*, *L. jensenii*, respectively, and the fifth one consisting of a mixture of anaerobes (11, 12). A second study in the U.S., this time in women with clinical BV, identified multiple VMB clusters consisting of highly diverse mixtures of anaerobic bacteria (13). These U.S. studies identified racial differences, with the highest prevalence of non-lactobacilli clusters in black women (11). The Rinda Ubuzima (RU) team was the first to describe the VMB of African women in a comprehensive manner (15). In a cohort of Rwandan women at high risk of acquiring HIV, we identified 6 VMB clusters, dominated by *L. crispatus*, *L. iners*, an anaerobe mixture with intermediate bacterial load, and anaerobe mixtures with high bacterial loads (three distinct clusters), respectively. We found significant trends in prevalence of HIV/STIs from low prevalence in the *L. crispatus* cluster, to higher prevalence in the *L. iners* cluster, and highest prevalence in the mixed anaerobe clusters. Until recently, molecular methods were not incorporated into large epidemiological studies with clinical outcomes due to limited availability and high costs. However, the Illumina MiSeq next generation sequencing platform (which is available in select laboratories in Europe but not in Rwanda; http://www.illumina.com/systems/miseq.ilmn) now makes high throughput sequencing possible and affordable. High throughput sequencing currently does not yet have any routine diagnostic value and is only used for research.

Even though evidence suggesting that not only symptomatic BV but also asymptomatic BV and intermediate microbiota cause long-term adverse outcomes is mounting, only symptomatic BV tends to be treated. BV is notoriously difficult to treat (16-18). About 60-80% of patients are cured after a standard course of antibiotics (typically oral or vaginal metronidazole or clindamycin) but recurrence rates are high: around 50% within 6 months (16). Two main reasons for this have been hypothesised: 1) the antibiotics not only reduce the abundance of BV-associated bacteria but also of healthy lactobacilli, which means that the VMB continues to be fragile after treatment; and/or 2) the antibiotics cannot penetrate the adherent biofilm that is often formed by the BV-associated bacteria, such as *Gardnerella vaginalis* (16). Antibiotic resistance does not seem to play a major role. BV tends to recur around the time of menstruation, which is thought to be due to declining oestrogen levels and/or declining vaginal pH due to menstrual blood flow, and after triggering events such as unprotected sex or vaginal douching. Many antibiotic, sexual behaviour, and vaginal hygiene interventions to reduce BV recurrences, and subsequently reduce adverse pregnancy outcomes, have been tried but with limited success thus far (reviewed in 16-19). None of these studies incorporated longitudinal molecular VMB assessments. TV is often associated with BV and the treatment for the two conditions is identical.

Therapies to restore and maintain a healthy VMB after antibiotic BV or TV treatment are currently not standard practice, but some clinicians in Europe and North America recommend twice weekly 0.75% metronidazole vaginal gel for 4-6 months to lower the risk of BV recurrence (16). This recommendation was tested in a randomised controlled trial in the U.S., which showed a statistically significant 57% reduction in the BV recurrence rate (20). Unfortunately, the effect disappeared after cessation of the intervention. However, since metronidazole can safely be used during pregnancy and has a high resistance threshold, this intervention could potentially be used in the second and third trimester of pregnancy in women at high risk of adverse pregnancy outcomes associated with an imbalanced VMB. Another study in American women at high risk of BV and STIs showed that metronidazole gel twice per week for 6 months significantly reduced BV and STI incidence in the intervention group (21). Two recent African trials evaluating oral (2g monthly) and vaginal metronidazole (5 nights every 3 months) for BV prevention showed significant beneficial effects, but the effects were modest, most likely due to the infrequent dosing (22, 23).

The World Health Organization (WHO) 2001 definition of probiotics is 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host'. Evidence is mounting that probiotic lactobacilli can restore and maintain lactobacilli-dominated VMB, are better able to disrupt BV-associated biofilms than antibiotics, and can be used safely for long periods without the risk of developing resistance (24). We have identified two vaginal probiotics that are licensed in Europe as medical devices and available over-the-counter: Ecologic Femi (Winclove Probiotics BV, Amsterdam, The Netherlands) and Gynophilus (Probionov, Clermont-Ferrand, France). While both vaginal probiotics contain lactobacilli with Qualified Presumption of Safety status in Europe (25), Ecologic Femi is a vaginal capsule containing multiple species of *Lactobacillus* and Gynophilus is a vaginal tablet containing one *Lactobacillus* species (together with its required nutrients) that was specifically designed to be released slowly over time. Clinical studies with Gynophilus in Austria, France, Russia and Poland thus far have shown beneficial VMB effects (27-30) and bothproducts are deemed safe (see section 11.1).

To summarise: current knowledge suggests that standard antibiotic treatment of BV alone is not sufficient in women with recurrent BV. The antibiotics also reduce the number of lactobacilli in the VMB, thereby leaving the VMB fragile. We believe that standard antibiotic treatment should be accompanied by behavioural counselling (safer sex and cessation of traditional vaginal practices), and perhaps also by promising vaginal antibiotic or probiotic VMB maintenance therapies.

3.2 Rationale

The RU team has worked with women at high risk of HIV and other STIs in Kigali since 2004 (31). We believe that interventions to reduce the extremely high prevalence and incidence of BV and other VMB imbalances are needed in this population, not only because BV is an important health problem in its own right, but also to prevent more serious consequences such as increased HIV/STI transmission and adverse pregnancy outcomes (all of which are also common in our study population). Our long-term goal is to conduct a randomised controlled efficacy trial of promising interventions (standard antibiotic treatment, followed by novel antibiotic and/or probiotic maintenance therapies, accompanied by behavioural counselling) to normalise the VMB for at least 6 months in order to reduce the incidence of BV, HIV/STIs, and adverse pregnancy outcomes. However, as a first step, we will conduct a pilot study to determine the feasibility of conducting such a trial, and to determine the longitudinal molecular effects of these promising interventions on the VMB of high risk women. These data are needed to choose the best interventions and to inform the design of the future efficacy trial.

4. STUDY DESIGN, OBJECTIVES AND ENDPOINTS

4.1 Study design

Sixty HIV-negative, non-pregnant, sexually active women aged 18-45 with BV by Amsel criteria and/or TV will be treated using oral metronidazole for 7 days. After successful treatment (defined as no BV by Amsel criteria and no TV on wet mount) and when they are free of vaginal candidiasis, other curable STIs and UTI, they will be randomised to 4 different VMB maintenance interventions (15 per group) within 3 days after completing oral metronidazole treatment:

- Group 1: Behavioural 'vaginal practices cessation and safer sex' counselling only (control);
- Group 2: Behavioural counselling plus 0.75% metronidazole vaginal gel, one applicator (5g) twice weekly for two months. If 0.75% metronidazole vaginal gel with stability data up to 30 °C cannot be obtained, we will use oral metronidazole 400-500mg twice weekly for two months (see section 11.1).
- Group 3: Behavioural counselling plus Ecologic Femi vaginal capsule, once per day for 5 days immediately after oral metronidazole treatment followed by thrice weekly, for two months

- Group 4: Behavioural counselling plus Gynophilus vaginal tablet, once every 4 days for two months.

In all 3 biomedical intervention groups, vaginal product use may be ceased temporarily during menstruation. Participants will be asked to adhere to the interventions for 2 months, and VMB assessments will take place before (screening and enrolment visits), during (Day 7, Month 1 and Month 2 visits), and after the interventions (Month 6 visit). Any episode of symptomatic BV by Amsel criteria, symptomatic vaginal candidiasis, STI (including TV) or UTI diagnosed during follow-up will be treated using standard oral therapies and the vaginal interventions will be allowed to continue during this treatment. A subgroup of 3 women in each study group (N=12) will be asked to self-collect vaginal swabs at home every Monday, Wednesday, and Friday for the first month of the intervention to allow for an in-depth evaluation of VMB adjustments to the initiation of the interventions (the 'frequent sampling subgroup'). These samples will be collected by a staff member, or dropped of by the participant, at least once per week at a time and location that is acceptable to the participant.

Primary clinical endpoints will be the safety and preliminary efficacy of the study products, the latter defined as incidence of BV by Amsel criteria, BV by Nugent score (Nugent 7-10), intermediate microbiota (Nugent 4-6), TV by culture and symptomatic vaginal candidiasis (by wet mount) during follow-up. Secondary clinical endpoints will be membership of specific VMB clusters over time as determined by clustering analysis of all available Illumina MiSeq data, the presence over time of individual VMB bacteria (as determined by Illumina MiSeq), and the incidence of STIs (HIV, syphilis, HSV-2, CT, and NG), and UTI (by urinalysis dipstick) during the follow-up period. In addition, we will conduct focus group discussions (FGDs), in-depth interviews (IDIs) and a quantitative survey to address specific feasibility objectives (see 4.2 below).

4.2 Specific objectives and endpoints

Primary clinical objectives	Endpoints
1. To determine the safety of the three biomedical interventions compared to the control group.	– Self-reported solicited and unsolicited (serious) adverse events (AEs) and social harms – Clinician-observed speculum exam findings
2. To determine the preliminary efficacy of the three biomedical interventions compared to the control group in reducing BV recurrence.	– BV by Amsel criteria, Nugent score 4-10, TV by culture and symptomatic vaginal candidiasis at any time during the intervention (Day 7, Month 1, Month 2) – BV by Amsel criteria, Nugent score 4-10, TV by culture, and symptomatic vaginal candidiasis after cessation of the intervention (Month 6) – Abnormal Nugent scores will be analysed as two categories (4-6 and 7-10) instead of one category (4-10) if statistical power permits.
Secondary clinical objectives	Endpoints
1. To determine which VMB changes at the molecular bacterial level are induced by initiation and cessation of the various interventions compared to the control intervention.	– Membership of specific VMB clusters (as determined by clustering analysis of all Illumina MiSeq data) over time – The presence over time of individual VMB bacteria as determined by Illumina MiSeq (and, if funding permits, other molecular methods), including the PPMs streptococci, staphylococci, and *E coli*. – The additional data points in the frequent self-sampling group will be analysed separately.
2. To determine preliminary	– Incidence of the following STIs between screening and

efficacy of the three biomedical interventions compared to the control group in reducing the incidence of STIs and UTI.	Month 6: HIV, HSV-2, syphilis, CT, NG, (and, if funding permits, human papillomavirus (HPV)) – Incidence of UTI between screening and Month 6
<u>Feasibility objectives</u>	<u>Endpoints</u>
1. To determine what Rwandan women know about BV and its impact on reproductive health outcomes.	– Quantitative survey with women attending recruitment and screening sessions, and FGD with women enrolled in the trial.
2. To determine acceptability of and adherence to the 4 interventions. [In the case of the control intervention, to determine if women who do not want to completely cease vaginal cleansing are willing to use clean water only in a manner that does not interfere with the biomedical interventions.]	– Quantitative survey, FGD and IDI data from women enrolled in the trial on acceptability and adherence. – Triangulation of quantitative and qualitative adherence data (see below). – The number of women withdrawing from the trial because of discontent with the interventions.
3. To determine the acceptability and feasibility of vaginal self-sampling at home three times per week for 4 weeks (with sample pick-up or drop-off).	– Quantitative survey and FGD data from women enrolled in the frequent self-sampling group on acceptability and feasibility. – The proportion of samples with sufficient DNA yield to generate Illumina MiSeq results.
4. To determine the required frequency of vaginal sampling in a future clinical trial.	– Using the above-mentioned primary and secondary clinical endpoints.
5. To determine the likely sustainability of the various interventions.	– Using the above-mentioned clinical and feasibility endpoints as well as cost data from this pilot study and IDI data from clinicians and policymakers.

If funding permits, we will also assess the following <u>additional secondary and tertiary clinical endpoints using stored specimens</u>: 1) additional VMB characterisation using other molecular methods such as quantitative PCR (qPCR) to quantify individual bacterial species, TV, and *Candida albicans* (secondary; addressing secondary objective 1 above); 2) HPV typing of endocervical specimens (secondary; addressing secondary objective 2 above); 3) urine microbiome characterisation by Illumina MiSeq and/or other molecular methods (tertiary), VMB biofilm characterisation(tertiary); and characterisation of the VMB and associated metabolites and immune responses in cervicovaginal lavages (CVLs; tertiary).

4.3 Sample size

This pilot study is descriptive and one of its aims is to inform sample size calculations of a future efficacy trial. Formal sample size calculations were therefore not performed. In the clinical trial, 60 women will be randomised into 4 groups consisting of 15 women each. Based on previous experiences with reproductive health clinical trials in the same study population, we expect to have to screen at least 120 women to enrol 60.

In the qualitative research, the number of FGDs or IDIs held will be governed by data saturation: no new discussions/interviews will be done when they no longer generate new information. The sample sizes listed in this study protocol are estimates based on our previous experiences with qualitative research. For feasibility objective 1, we expect to conduct quantitative face-to-face interviews with at least 30 women attending recruitment sessions who did not pass pre-screening (in addition to the approximately 120 women attending screening visits) and conduct 2 FGDs with women enrolled in

the trial. For feasibility objective 2, we expect to conduct 2 FGDs and 5 IDIs (in addition to quantitative face-to-face interviews with the 60 women enrolled in the trial). For feasibility objective 3, we expect to conduct one FGD with all 12 women in the frequent sampling subgroup. For feasibility objective 5, we expect to conduct 5 IDIs with clinicians and policymakers. The total number of FGDs and IDIs will therefore be approximately 5 FGDs and 10 IDIs.

4.4 Randomisation and blinding

Participants will be randomised at the enrolment visit. Study group allocation will be concealed until the participant was confirmed to be eligible and has provided written informed consent for enrolment. Computer-generated group allocations will be generated prior to study initiation and concealed in randomisation envelopes numbered 1-60. The envelopes will be opened in consecutive order as new participants are randomised. The study clinicians cannot be blinded because the study products look different and dosing schedules are different. However, we will blind the laboratory technicians at RU and at UoL so that the assessment of all laboratory endpoints is blinded. This will be achieved by ensuring that study samples and accompanying documents that are shared with the laboratory technicians do not contain any information about the study arm that the participant was randomised to.

5. STUDY POPULATION

5.1 Target population

Our target population is HIV-negative, non-pregnant, sexually active women between 18-45 years at high risk of HIV/STIs/BV in Kigali, Rwanda. RU has worked with this target population for 10 years (15, 31). The HIV prevalence in a previous study in this population was 24% (31), the BV prevalence 40% (15), and the TV prevalence 17% (31). The HSV-2 and HPV status of participants will be determined (the latter only if funding permits; see below), and will be considered for inclusion in statistical models as a confounding variable, but will not be used to select women for enrolment. We decided to limit inclusion to women of reproductive age (18-45 years) because our long-term goal is to identify an efficacious intervention that can subsequently be tested in women of reproductive age with high risk of HIV/STIs/BV and/or pregnancy. An intervention that is efficacious in women of reproductive age is not necessarily efficacious in postmenopausal women due to expected VMB changes after menopause.

5.2 Eligibility criteria

Inclusion criteria:

- Female, 18-45 years old
- Sexually active, defined as having had sex at least twice in the two weeks prior to screening
- At high risk of HIV/STIs/BV, defined as having had more than one sexual partner in the last 12 months OR having been treated for an STI and/or BV in the last 12 months
- Successfully treated for BV by Amsel criteria or TV (defined as no BV by Amsel criteria and no TV on wet mount at the enrolment visit) and free of HIV (at screening), pregnancy (at screening and the enrolment visit), symptomatic vaginal candidiasis and UTI (at the enrolment visit), and syphilis, NG, and CT (at the results visit). Due to the long turn-around time for NG and CT lab test results, women may be enrolled when their NG and CT results are not yet known, but a new participant will be randomised for each woman whose screening sample comes back positive for NG and/or CT after having been randomised. The NG/CT-positive women will be allowed to complete the study but their data may be removed from some analyses.
- Currently in good physical and mental health as judged by a study physician
- Willing and able to adhere to study procedures and provide written informed consent.

According to Rwandan ethics guidelines, unmarried women aged 18-20 also need the written informed consent of a parent or guardian.
- Women are allowed to use contraception, including hormonal contraception; use of these methods will be taken into account in the instructions given to women about probiotic use and in the data analysis.

Exclusion criteria:
- HIV positive
- Pregnant
- Clinician-observed genital ulcers, condylomata, or other genital abnormalities at screening and/or enrolment
- Underwent a gynaecological surgery/invasive procedure in the 3 months prior to screening
- History of significant urogenital prolapse, undiagnosed vaginal bleeding, urine or faecal incontinence, or blood clotting disorders
- Allergic to metronidazole or any other components of the study drugs
- Not willing to stop use of other oral or vaginal probiotics from the screening visit until the end of study participation
- Participating in another health intervention study
- For any other reason potentially interfering with participant safety or protocol adherence as judged by the Principal Investigator (these reasons will be recorded)

5.3 Recruitment

RU will use its network of community mobilisers to organise recruitment meetings in the Kigali neighbourhoods where recruitment for previous RU studies with high risk women took place. These neighbourhoods are Gikondo, Kimihurura, Remera and Muhima. 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. During recruitment meetings, study staff will explain the study to potential participants, and there will be sufficient time for questions and answers. At the end of the meeting, study staff will check potential eligibility of individual women who are interested in participating using an anonymous checklist (referred to as pre-screening). No individual data will be recorded during this pre-screening process; only overall statistics (number of women who attended the recruitment session, were pre-screened, and were deemed eligible after pre-screening) will be kept for each recruitment session. Women who are interested and deemed potentially eligible at pre-screening will be asked for contact information (this information cannot be linked to the pre-screening checklist) and will be invited to attend a screening visit at the RU research clinic in Kiyovu, Kigali.

5.4 Retention and withdrawals

Study staff, with the help of community mobilisers, will make every effort to retain participants to minimise possible bias associated with loss-to-follow-up. All participants are required to provide contact information for herself and at least one other contact person at screening, and this information will be verified at each subsequent visit; they will consent to this during the screening and enrolment informed consent processes, respectively. Study staff will ask each participant for permission to contact her and her contact person(s) in specific ways, such as by mobile phone and/or home visit. If a participant misses a visit, study staff will attempt to contact her in accordance with what was agreed. All contact attempts will be documented in the participant's records.

Visit windows for the study visits are as follows:
- Screening + results visits: the entire screening process should take no longer than 6 weeks

- Enrolment visit: maximum of 3 days after completion of oral metronidazole treatment
- Day 7: Day 5 – Day 9
- Month 1: ± 7 days
- Month 2: ± 7 days
- Month 6: - 7 days

If a participant misses a visit window, this will be considered non-adherence to the visit schedule but not a protocol deviation. A participant will be considered lost to follow-up only at study close-out after multiple contacts by staff (up to a maximum of three face-to-face contacts per missed visit) to complete the participant's visit schedule have been unsuccessful.

Participants may voluntarily withdraw from the study for any reason at any time during the study. If a participant does withdraw from the study, study staff will attempt to complete any outstanding evaluations of the participant (see 'withdrawal procedures') but only if the participant agrees. The reason(s) for the withdrawal will be recorded in the participant's records. The Principal Investigator may also discontinue participants (for example, if this is deemed in the best interest of the participant) but will first ask the Chief Investigator for a second opinion. Women who become pregnant or acquire an STI (including HIV) during the study may continue. Participants who are discontinued from the study cannot be rescreened.

6. STUDY PROCEDURES - CLINICAL

At all study visits, curable genital infections (syphilis, NG, CT, TV, symptomatic BV, and symptomatic vaginal candidiasis) will be treated at RU free of charge and partner notification and treatment services will be offered if applicable. 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 Visit flow chart and schedule of assessments

See Annex 1 and 2.

6.2 Screening

The screening process is in at least two study visits (screening and results visit) and should take no longer than 6 weeks.

Upon arriving at the RU research clinic all potentially eligible participants will be assigned a participant identification number after which the screening informed consent process will be done according to existing RU standard operating procedures. It will include a group educational session, followed by an individual session consisting of a formal assessment of literacy, a discussion about the study with a research nurse, a formal assessment of understanding of the study, and the actual signing of the consent form (see 10.5 for details).

After written informed consent has been obtained, the following procedures will take place:
1. Collection of contact information;
2. Completion of screening eligibility checklist. Women who are menstruating at the time of this

visit will be asked to return for completion of screening procedures after they have finished menstruating.

3. Face-to-face interview (sociodemographic characteristics, sexual behaviour, vaginal practices, circumcision status and penile hygiene of male partners, medical and reproductive history with an emphasis on STIs and recurrent reproductive tract infections, current medications and contraception, current genital symptoms, and BV knowledge);
4. HIV pre-test and HIV/STI/BV risk reduction counselling;
5. Collection of blood (10 ml; for HIV, HSV-2 and syphilis testing) and urine (for pregnancy and urinalysis dipstick testing);
6. Rapid HIV, pregnancy and urinalysis dipstick tests in the onsite RU laboratory;
7. Post-HIV test counselling, provision of rapid test results, and provision of treatment (for UTI on urinalysis dipstick) and referrals (for HIV and pregnancy) as needed.
8. If HIV-positive and/or pregnant: not eligible for enrolment. A speculum examination will only be done when clinically indicated but women will be asked to donate vaginal swabs for molecular VMB testing. These swabs will be collected without speculum insertion, and can be collected by the clinician or by the woman herself depending on her preference.
9. If HIV-negative and not pregnant: Speculum examination with visual inspection and specimen collection in the following order: 1) vaginal swabs for vaginal pH measurement, wet mount, Gram stain Nugent scoring, TV culture, and VMB assessments (see 8.2); 2) endocervical swabs for NG/CT PCR and HPV typing; and 3) CVL for additional VMB assessments (see 8.2).
10. Wet mount microscopy (Amsel criteria, *Candida*, and TV) in the onsite RU laboratory;
11. Provision of treatment as follows:
    - If only BV by Amsel criteria and no/minor vaginal symptoms: postpone treatment until the results visit 1-2 weeks later. According to local treatment guidelines and the World Health Organisation, asymptomatic BV does not have to be treated. However, in this study, all women with BV by Amsel criteria will be treated because we want to achieve a healthy VMB in all women. However, postponing treatment to allow for other diagnostic testing to be completed first will not jeopardise that goal.
    - If BV by Amsel criteria and significant vaginal symptoms: start oral metronidazole for 7 days, and schedule a results visit for within 3 days after completion of this treatment. This will give the team 10 days to obtain all other STI test results, which will determine final eligibility for enrolment (see 6.3.2).
    - If TV on wet mount: start oral metronidazole for 7 days, and schedule a results visit for within 3 days after completion of this treatment. This will give the team 10 days to obtain all other STI test results, which will determine final eligibility for enrolment (see 6.3.2).
    - If vaginal candidiasis and vaginal symptoms (regardless of BV or TV): start standard antifungal treatment and repeat screening after treatment (see 6.3.2).
12. Reimbursement, and scheduling of the results visit in 1-2 weeks for all HIV-negative, non-pregnant women regardless of eligibility up to that point (allowing sufficient time to complete all diagnostic laboratory testing);
13. Participant leaves the RU clinic;
14. Further local laboratory testing:
    - Blood specimens: HSV-2 and syphilis serology
    - Vaginal specimens and CVL: TV culture, and initial processing and storage of samples for Gram stain Nugent scoring at RU and VMB and biofilm assessments at UoL (see 8.2)
    - Endocervical specimens: NG/CT PCR, and initial processing and storage of one swab for HPV typing in Europe (if funding permits; see 8.2). We will only conduct HPV typing if funding permits as this test is not available in Rwanda and does not have any therapeutic consequences for women.

6.3 Results visit

All HIV-negative, non-pregnant women regardless of eligibility up to that point will be asked to return to the study clinic 1-2 weeks after the screening visit to obtain their screening test results.

The following procedures will take place at the results visit:

1. Confirmation of contact information;
2. Provision of HSV-2, syphilis, NG, CT, and TV culture results.
   - If syphilis, NG or CT positive or received treatment for candidiasis at screening (regardless of other test results): Treat STIs according to national guidelines and ask the woman to return to RU as soon as possible after completing her STI treatment for a repeat wet mount. Women who received candidiasis treatment at screening can provide a repeat wet mount at this visit. Vaginal swabs to measure vaginal pH and prepare a wet mount (as well as a slide for Gram stain Nugent scoring) will be obtained without speculum. If negative for BV by Amsel criteria and TV on wet mount, the woman is not eligible for enrolment. If positive for BV by Amsel criteria and/or TV on wet mount, treat using oral metronidazole for 7 days and schedule the enrolment visit within 3 days of completing treatment.
   - If TV culture positive and not yet treated for TV (based on wet mount) at screening: treat using oral metronidazole for 7 days and schedule the enrolment visit within 3 days of completing this treatment.
   - If BV by Amsel criteria at screening and not yet treated (regardless of symptoms): treat using oral metronidazole for 7 days and schedule the enrolment visit within 3 days of completing this treatment.
   - A positive HSV-2 result will not have any treatment or eligibility consequences.
3. Due to the logistics of NG/CT PCR, we expect long turn-around time. For this reason, and because the prevalence of NG and CT is much lower than the prevalence of the other STIs, women may be enrolled when their NG and CT results are not yet known. However, a new participant will be randomised for each woman whose screening sample comes back positive for NG and/or CT after having been randomised. The NG/CT-positive women will be allowed to complete the study but their data may be removed from some analyses.
4. Due to the logistics of Nugent scoring, Nugent scores may not be available at the enrolment visit. Results will not be used to guide enrolment and treatment decisions anyway, but will only be used in the data analysis.
5. Confirmation of eligibility thus far using the enrolment checklist. The screening process may be repeated up to 3 times.

6.4 Enrolment visit

The enrolment visit will be scheduled as soon as possible, and within a maximum of 3 days after completion of oral metronidazole treatment. This window of 3 days is needed in case the last day of oral metronidazole treatment is in the weekend or during menses. At the enrolment visit, the following procedures will take place:

1. Urine pregnancy and urinalysis dipstick testing. If pregnant, not eligible. If UTI, treat and do not yet randomise. Repeat wet mount (and collect Gram stain for Nugent scoring) and enrol based on new wet mount results as in 6.3.2.
2. Enrolment informed consent procedures;
3. Face-to-face interview (update on current medications, contraception and genital symptoms and baseline assessment of AEs and social harms)
4. Speculum examination with collection of vaginal swabs for vaginal pH measurement, wet mount, Gram stain Nugent scoring, TV culture and VMB assessments (see 8.2); endocervical swabs to store for potential NG/CT testing (if required for clinical reasons or for VMB interpretation); and a CVL to store for additional VMB assessments (see 8.2). If BV by Amsel

criteria, TV on wet mount or symptomatic vaginal candidiasis on wet mount, treat and do not yet randomise. Repeat wet mount (and collect Gram stain for Nugent scoring) and enrol based on new wet mount results as in 6.3.2.

5. Randomisation;
6. HIV/STI/BV risk reduction counselling plus explanation of the intervention that the participant was randomised to (including detailed explanations of potential side effects, and in the case of metronidazole, potential side effects when metronidazole is combined with alcohol use), provision of study products, and application of the first dose of the relevant intervention under supervision of a study staff member (and practice of vaginal self-sampling if in the frequent sampling group). Women will be asked to stay in the study clinic for a minimum of about 30 min after application of the first dose to identify and treat any immediate allergic reactions (these are exceedingly rare).
7. Reimbursement, and scheduling of the Day 7 visit;
8. Participant leaves the RU clinic;
9. Further laboratory testing:
   - Vaginal swabs and CVL: Initial processing and storage of a vaginal Gram stain for Nugent scoring at RU, and vaginal swabs and CVL for VMB and biofilm assessments at UoL (see 8.2);
   - Endocervical swabs: Initial processing and storage for potential local NG/CT testing;
   - Urine specimens: Initial processing and storage for urine microbiome analyses in Europe (if funding permits; see 8.2).

Use of Amsel criteria: As noted in the introduction, one of the four Amsel criteria is the presence of vaginal discharge, and we may therefore miss asymptomatic cases of BV if we use the Amsel criteria instead of Nugent scoring. This is not a problem for the women themselves because asymptomatic BV is normally not treated, but it is a missed opportunity for this study. We will closely monitor our Amsel data in the beginning of the study (compare the Amsel data with the Nugent data), and if warranted, we may decide to only use the three laboratory Amsel criteria to guide enrolment and treatment decisions. In that case, BV by Amsel criteria would be defined as 2 of the 3 laboratory criteria throughout this protocol.

6.5 Follow-up visits Day 7, Month 1 and Month 2

Follow up visits in the clinical trial will take place at Day 7 (7 days after start of the intervention), Month 1, Month 2 (cessation of the intervention), and Month 6 (4 months after cessation of the intervention).

The following procedures will take place during the Day 7, Month 1 and Month 2 follow up visits:

1. Confirmation of contact information. Women who are menstruating at the time of a study visit will be asked to return for completion of visit procedures after they have finished menstruating.
2. Face-to-face interview (update since the last study visit on: current medications, contraception, genital symptoms, sexual behaviour, vaginal practices, penile hygiene practices of male partners, having been diagnosed and/or treated for STIs/BV/vaginal candidiasis or other medical conditions, adverse events/social harms, and acceptability of and adherence to the interventions).
3. Speculum examination with collection of specimens: vaginal specimens for vaginal pH, wet mount, Gram stain Nugent scoring, TV culture and VMB and biofilm assessments at UoL, and a CVL to store for additional VMB assessments at UoL. If the participant has signs or symptoms of a reproductive tract infection, specimens will be collected for diagnostic testing as described for the screening visit.
4. Counselling with as main aim to address any questions or concerns the participant may have about her intervention. At the Month 2 visit, the intervention will be terminated.
5. Reimbursement, and scheduling of the next visit;

6. Participant leaves the RU clinic;
7. Further laboratory testing:
   - Diagnostic specimens (only in the case of genital signs or symptoms): see screening visit
   - Initial processing and storage of vaginal Gram stains for Nugent scoring at RU, and vaginal swabs and CVLs for VMB and biofilm assessments at UoL.

6.6 Final Month 6 visit

The following procedures will take place during the Month 6 visit:
1. Confirmation of contact information. Women who are menstruating at the time of this visit will be asked to return for completion of visit procedures after they have finished menstruating.
2. Face-to-face interview (the same topics as the other follow up visit interviews but with an emphasis on overall evaluation of the feasibility and acceptability of the interventions)
3. Pre-HIV test and HIV/STI/BV risk reduction counselling
4. Collection of blood (10 ml; for HIV, syphilis and HSV-2 testing, the latter only if negative at baseline) and urine (for pregnancy and urinalysis dipstick testing)
5. Speculum examination with specimen collection in the following order: 1) vaginal swabs for vaginal pH measurement, wet mount, Gram stain Nugent scoring, TV culture, and VMB and biofilm assessments at UoL; 2) endocervical swab for NG/CT PCR and HPV typing (if funding permits); 3) CVL for additional VMB assessments at UoL.
6. Rapid HIV, pregnancy, urinalysis dipstick, and wet mount (Amsel criteria, *Candida* and TV) testing in the onsite RU laboratory
7. Post-HIV test counselling, provision of rapid test results, and treatment and referrals as needed
8. Participant leaves the RU clinic. She will be told that she will be contacted should any of the other diagnostic test results come back positive.
9. Further local laboratory testing:
   - Blood specimen: syphilis and HSV-2 serology;
   - Vaginal specimens and CVL: Nugent scoring, TV culture, initial processing and storage for VMB and biofilm assessments at UoL;
   - Endocervical specimen: NG/CT PCR, HPV typing (if funding permits);
   - Urine specimen: Initial processing and storage for urine microbiome assessments in Europe (if funding permits).

6.7 Frequent sampling group

During the enrolment informed consent procedures, women will be asked if they are willing to take part in the frequent sampling procedures on top of regular study procedures should they be selected. Only 3 women per randomisation group (total N=12) will be selected. The actual selection of women will be guided by the overall study visit schedule to ensure that the study staff responsible for specimen pick-up at the participants' homes and the laboratory teams can cope with the additional workload. Women in the frequent sampling group will undergo regular study procedures, but in addition, will be asked to collect two vaginal swabs on Monday, Wednesday and Friday morning at home during the first month of the intervention. Sample collection may continue during menses. Women will be taught how to self-sample by RU study staff at their enrolment visit. All self-collection materials will be provided to the participant in such a manner that hygienic storage at home is possible. Participants will be asked to store the specimens in a refrigerator or a cool place away from direct sunlight. The study team assumes that most women will not have access to a refrigerator and will therefore provide storage media that will protect the samples from degradation. Specimens will be picked up by study staff, or dropped off by study participants, on a weekly basis (or more often if feasible) and will be processed and stored by the RU laboratory immediately after receipt. The exact pick-up/drop-off schedule will be negotiated with each participant individually to ensure that it is feasible and acceptable to them.

To preserve confidentiality and prevent social harms, the following procedures will be followed:

- Only women who are enrolled in the study will be asked whether or not they are interested in participating in the self-sampling component of the study.
- Only women who are interested in participating in the self-sampling component will be considered for selection.
- The selected participants can freely choose their preferred collection method: home visit by study staff or sample drop-off.
- Drop-off sites will be selected with the help of our outreach team, using stringent criteria to preserve the participant's confidentiality and ensure easy access. The locations we used in previous studies were rooms in health centres or one stop centres close to the participants' homes.
- Home-visits will only be conducted with permission from the participant by our female outreach staff who have been thoroughly trained in how to preserve confidentiality. These female staff will always be accompanied by a male driver, who has also been trained in how to preserve confidentiality. RU cars do not display any logos or project identifiers and RU staff do not wear uniforms.

6.8 Unscheduled visits

All participants will be encouraged to visit the RU clinic in between study visits when they experience SAEs, AEs that they suspect are product-related, or new episodes of STIs, BV or vaginal candidiasis. Study staff may ask participants to return to the RU clinic in between regular study visits if diagnostic tests come back positive and require counselling, partner notification, treatment and/or referral. Other unscheduled visits may take place at the request of participants or study staff at any time during the study. All unscheduled visits will be documented.

6.9 Withdrawal procedures

Women who choose to withdraw during the first 2 months of study participation will be asked if they are willing to answer a few questions (focusing on reasons for withdrawal, acceptability of and adherence to the intervention, adverse events/social harms and current genital symptoms) and to provide vaginal swabs (without insertion of a speculum) for VMB assessments. Women who choose to withdraw between the Month 2 and Month 6 visits will first be asked if they are willing to complete all Month 6 procedures at the time of withdrawal, and if not, if they are willing to answer a few questions and provide vaginal swabs (without insertion of a speculum) for VMB assessments. Only the procedures that the participant agrees to will be implemented.

7. STUDY PROCEDURES – SOCIAL SCIENCE

As mentioned in section 4.3 (sample size), a total of about 5 FGDs and 10 IDIs will be conducted to address the feasibility objectives. In addition, we expect to conduct quantitative face-to-face interviews focusing on BV knowledge with at least 30 women attending recruitment sessions (using similar questions that will be included in the screening interview, which will be completed by approximately 120 women during screening visits).

At the recruitment sessions prior to the recruitment presentation, women will be asked if they are willing to be interviewed for about 15 min about their BV knowledge (the recruitment presentation contains explanations about BV and might therefore bias the interview results). If they say yes, study staff will explain the interviewing procedures to them, will give them an information sheet, and will ask for verbal consent. No identifying or contact information will be recorded, and the interview will

be conducted outside of hearing distance of others present at the recruitment site. After the interview, the pre-screening eligibility checklist will be administered (again, anonymously) so that we know whether the woman is potentially eligible for the trial or not. Only the overall outcome of that assessment will be written on the person's anonymous questionnaire. After the interview, participants will be given a small token of appreciation, such as a bar of soap or hand lotion. They will not be given a monetary reimbursement. Interviews will be conducted until a total N of 30 has been reached.

At screening visits, all women will be asked if they would be willing to participate in FGDs or IDIs at a later date should they be selected. Only women who said yes may be approached by study staff. Selected women will be asked to provide separate informed consent for each FGD and/or IDI that they participate in. All FGD and IDI procedures with study participants will take place at the RU research clinic. The FGDs/IDIs on acceptability and adherence will be held with participants who have completed their 2-month intervention period, the FGD with the frequent self-sampling group after all 12 women have completed the frequent self-sampling procedures, and the other FGDs/IDIs at any time during the data collection period.

Approximately 5 IDIs will be conducted with clinicians and health policymakers. 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.

8. STUDY PROCEDURES - LABORATORY

8.1 Diagnostic tests

The following diagnostic tests will be performed in the onsite RU laboratory (or at the National Reference Laboratory in Kigali if needed) using validated test kits and standard operating procedures:

- Urine hCG pregnancy test
- HIV by rapid test algorithm based on the most recent Rwandan HIV voluntary counselling and testing guidelines
- Syphilis by RPR and *Treponema pallidum*-specific test
- HSV2 by serology
- CT and NG by PCR (at the National Reference Laboratory in Kigali)
- TV by wet mount microscopy and InPouch culture
- Vaginal candidiasis by wet mount microscopy
- BV by Amsel criteria and Gram stain Nugent scoring
- UTI by urinalysis dipstick test
- If funding permits, the presence of HPV types (and particularly oncogenic HPV types) will be determined using molecular methods in a specialised laboratory in Europe. This test is not available in Rwanda and does not have any therapeutic consequences for Rwandan women.

8.2 Research tests

Most testing for research purposes will be conducted at UoL, Liverpool, UK. Specimens will be processed and stored (at -80°C, or -20°C if -80°C is not possible) at RU and shipped in batches from RU to UoL using RU standard operating procedures (which are based on local and international shipping regulations) and Material Transfer Agreements. They will be stored at -80°C at UoL immediately after arrival until testing.

- DNA will be extracted from vaginal swabs (and/or CVLs), bacterial DNA will be amplified using primers directed to highly conserved regions of the 16S rRNA gene, and bacteria will be characterised using Illumina MiSeq sequencing of variable regions of the 16S rRNA gene. These procedures were adapted from Fadrosh et al (32) and have been successfully used in previous studies by UoL.
- If funding permits, individual VMB bacteria (commensals, PPMs, and probiotic strains), TV and *Candida albicans* may be further investigated using qPCR or other molecular methods.
- If funding permits, vaginal fluid on microscopy slides may be evaluated for biofilm.
- If funding permits, CVLs may be used to characterise bacterial metabolism (by assessing the presence of bacterial metabolites or proteins in the CVLs) and host immune responses (by identifying the presence of cytokines, other inflammatory markers, and human proteins in the CVLs).
- If funding permits, urine samples will be analysed by Illumina MiSeq at UoL to characterise the urine microbiome and compare this to the VMB of the same women.

9. DATA MANAGEMENT AND ANALYSIS

We will use paper-based source documents and electronic case report forms (eCRFs). All study documents will be kept at RU in lockable cabinets and/or rooms for confidentiality reasons. Documents containing names and/or signatures will be kept separately from all other study documents containing identification numbers.

The electronic study participant identification register (eSPIR; containing participant names, identification numbers, contact information, contact attempts, and visit attendance) and clinical trial database (containing all CRF data) will be programmed by specialised clinical database programmers. Data will be entered into the eSPIR by the RU receptionist when the participant first enters the RU research clinic at each study visit. CRF data will be double-entered into the clinical database by RU data entry clerks after initial monitoring for CRF completeness and accuracy has been completed. FGDs and IDIs will be tape-recorded, transcribed verbatim, translated into English, and analysed in Atlas Ti software. The SPIR, clinical database, transcripts, and Atlas Ti data will be kept on password-protected RU computers and access will be limited to key project staff. Data will be backed-up regularly at RU, and will be shared with the UoL Chief Investigator at regular intervals for safekeeping at UoL. The RU standard operating procedures related to data entry and management, and the UoL data safety and privacy guidelines, will be followed. The molecular VMB data and other laboratory research data will be managed separately at UoL. These data will only contain participant identification numbers, and UoL laboratory staff will not have access to the SPIR or any other personal identifying information.

The analysis of primary and secondary clinical outcomes will be conducted by the UoL Chief Investigator or designee(s) in close collaboration with the Principal Investigator. These analyses will be conducted in accordance with a priori developed statistical analysis plans, which will be circulated to all co-investigators and external advisors for review and input. The feasibility outcomes will be analysed by the RU social science team led by Jennifer Van Nuil.

10. ETHICAL ISSUES AND PROCEDURES

10.1 Ethical review

The protocol will be reviewed by the Principal Investigators, co-investigators, and trial steering committee (TSC) members and approved by the Chief Investigator and Principal Investigator. Prior to

enrolment of the first participant, the protocol will be approved by the Rwanda Biomedical Centre 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 (RESPI).

10.2 Risks and benefits

Physical burdens may include discomfort in the vagina during collection of vaginal and endocervical swabs or use of the vaginal products and/or bruising at the site of venipuncture. Psychological burdens may include embarrassment during the face-to-face interview and pelvic exam 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), especially at the screening and Month 6 visits.

Participants will receive their HIV test results according to the national HIV voluntary counselling and testing guidelines. Pregnant women will be referred to an antenatal clinic where they will receive more comprehensive care than we can offer them. Although a positive HSV-2 or HPV status does not lead to medical intervention, clients who test positive for HSV-2 or high-risk HPV will be informed of their result. They will be educated about HSV-2, and told to seek care immediately (as well as use condoms) if they experience an active herpes outbreak. They will also be educated about HPV, the link between HPV and cervical cancer, and how best to prevent HPV infections.

Benefits for all participants include free HIV counselling and testing, and free diagnosis and treatment of several common STIs, BV, and vaginal candidiasis. Participants who suffer from recurrent BV may experience clinical benefit from the study interventions. 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 throughout the study.

10.3 Compensation and insurance

Women will be compensated in the amount of 3,000 Rwandese francs (the equivalent of 3 GBP) per visit at the RU study clinic that is scheduled by study staff. They will not receive monetary compensation for unscheduled visits at their own initiative but will receive the benefits described under 10.2.

UoL will obtain a 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 eSPIR). 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 FGDs, participants will be told they can use a name other than their given name for confidentiality. Transcripts from the FGDs and/or IDIs will not contain participants' given names.

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, particularly when conducting home visits for participant tracing purposes. 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 (as determine by formal literacy assessment) 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.

Four separate participant information sheets (PIS) and informed consent forms (ICF) will be used: one for screening, one for enrolment, one for FGDs and one for IDIs. A fifth PIS will be given to women participating in the BV interview at recruitment sites but we will obtain verbal consent from them given the anonymous nature of the short interview (15 min) and lack of any other study procedures. In the clinical trial (screening and enrolment visits), the informed consent process will start with a group educational session, followed by an individual session attended by the participant and, if applicable, her parent/guardian and/or her witness. The individual session consists of a formal assessment of literacy, a discussion about the study with a research nurse counsellor, a formal assessment of understanding of the study, and the actual signing of the consent form. The FGDs will only be done with participants who have been enrolled in the trial for a while. Study staff will therefore know them well, and may skip the formal assessments of literacy and understanding if they feel confident about the participant's literacy and understanding of the study. In the case of IDIs with clinicians and policymakers, the informed consent process will be individual, and will not be accompanied by formal assessments of literary and understanding because the interviewees are highly educated professionals.

All consent procedures (including the literacy assessment and assessment of understanding) 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.

During the screening and enrolment consent process, participants will be asked to sign separately for long-term storage of specimens for the future testing that has already been specified in this study protocol (tertiary objectives) or for closely related additional VMB research. Any additional research that is not closely related to the study objectives in this protocol would be formally approved by the study sponsor and the ethics committees listed in secion 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.

11. INVESTIGATIONAL PRODUCTS

11.1 Study products

The active ingredient in metronidazole oral tablets and vaginal gel is the antibiotic metronidazole.

For the initial treatment of BV and TV, RU uses generic metronidazole oral tablets that are readily available in local licensed pharmacies in Kigali (for an example, see the package insert for Eflaron in Appendix 15.3). These generic tablets were approved by the Rwandan Government and are stable at temperatures up to 30 °C. The 0.75% metronidazole gel MetroGel-Vaginal (Graceway Pharmaceuticals, Bristol, TN, USA) is approved by the US Food and Drug Administration (FDA) and is also stable at temperatures up to 30 °C (see US FDA Prescribing Information in Appendix 15.3). Each gram of gel contains 7.5mg metronidazole, 0.8mg methylparaben, 0.2mg propylparaben, in a gel consisting of purified water, propylene glycol, carbomer 934P and edentate disodium. The gel is formulated at pH 4.0 and comes in a 70g aluminium tube with 5 disposable applicators. It is administered into the vagina using a disposable applicator (which has to be filled by the user), and 5g of gel is administered with each application. MetroGel-Vaginal is not available in Rwanda and will be imported from the USA by RU for use in this study only. It can be used for BV treatment, but as described in the introduction, some physicians also use it as a maintenance therapy to prevent BV recurrence in the same dosing schedule as we are proposing to use in this study (16, 20). If RU is not able to import MetroGel-Vaginal gel, we will use metronidazole oral tablets (the same generic tablets as described above) 400-500mg twice per week for 2 months instead. These tablets have been approved for prophylaxis of anaerobic infections.

The other two study products are vaginal probiotics. In Europe, vaginal probiotics are typically registered as medical devices; this applies to the probiotics to be used in this study as well. The Notified Bodies do not consider them medicines because they are applied to a body surface but not absorbed by the body. The Dutch Notified Body explains this as follows: 'The device obtains its intended action by enhancing the natural vaginal flora by preventing or alleviating any imbalance in this flora. It achieves this principally by promoting the formation of lactic acid, the production of hydrogen peroxide, and competition with potential pathogens for space and substrate. This action is not considered to be falling under pharmacological, immunological or metabolic means; it does not directly act on the human body through the actions mentioned.' Furthermore, the vaginal probiotics to be used in this study only contain bacterial strains with Qualified Presumption of Safety status (23), have CE marketing approval in Europe, and are produced in facilities with current Good Manufacturing Practice (cGMP) certificates. See Appendix 15.3 for the package inserts.

Ecologic Femi (Winclove Probiotics, Amsterdam, The Netherlands) is a vaginal capsule containing multiple lactic acid-producing bacteria (mainly lactobacilli). These bacteria include *Bifidobacterium bifidum* W28, *L. acidophilus* W70, *L. helveticus* W74, *L. brevis* W63, *L. plantarum* W21, and *L. salivarius* W24. They were selected for their ability to produce lactic acid and hydrogen peroxide, adhere to epithelial cells, and inhibit growth of *C. albicans*. Ecologic Femi was found to be stable at 25°C and 30°C for up to 2 years. The dosing schedule that we propose to use in this study has been approved by Winclove and the Dutch Notified Body.

Gynophilus LP vaginal tablets (Probionov, Clermont-Ferrand, France) were designed for controlled release of lactobacilli in the vagina. Each Gynophilus tablet contains 876.9 mg of Lcr Regenerans, a culture of the *Lactobacillus casei rhamnosus* 35 (Lcr35) strain specially designed for the vaginal environment. It has a long history of use in gynaecology (more than 30 years) and an excellent safety profile (clinical trials for vaginal use of this strain have been conducted and an Investigator's Brochure is available). Lcr Regenerans' ability to ferment glycogen to lactic acid is five times superior to that of the native Lcr35 strain; 95% of the lactic acid produced by Lcr35 is L-lactate, corresponding to the lactic acid naturally present in the vaginal cavity. A tablet of Lcr Regenerans contains not only the probiotic strain but its culture medium as well. The tablet disintegrates in the vagina and forms a gel. The tablets are stable at 25°C and 30°C for up to 10 months (stability testing of the tablet is ongoing and the same strain in a capsule is stable up to 2 years). The dosing schedule that we propose is as recommended by Probionov and approved by the French Notified Body.

11.2 Drug regulatory authorities

Since all study products are available on the market in Europe (vaginal probiotics), the USA (Metrogel-Vaginal), or Rwanda (generic metronidazole oral tablets), no regulatory approval is needed for this study protocol. However, the study products are currently not available in Rwanda (with the exception of generic metronidazole oral tablets), and we will therefore obtain import permits from the Rwanda Minister of Health and the Ministry of Health Pharmacy Task Force prior to shipment of the study products to Rwanda. Details about this procedure are available in the RU SOP for Investigational Medicinal Product Importation.

11.3 Study product accountability and packaging

Metronidazole oral tablets and vaginal gel, Ecologic Femi capsules and Gynophilus tablets can be kept at room temperature, away from direct sunlight exposure Study products will be shipped in their commercial packaging from Europe or the USA to Rwanda, and will be kept in the on-site pharmacy room at RU after arrival in Rwanda. The temperature of this onsite pharmacy room is monitored and recorded every work day. The RU study product accountability SOPs will be followed. Participants will receive a sufficient amount of study product from the RU pharmacy to bridge the time period between study visits, up to the upper visit window. The study products will be given to participants in their original commercial packaging. In addition, a study leaflet with product and use information will be provided to each study participant in a language that is understandable to her. Participants will be asked to bring the packaging of used products as well as unused study products with them to the RU clinic at each study visit. Study staff will record study product dispensed, used and returned unused at each study visit. All unused study products that are left-over at study close-out, as well as the packaging of used products returned to RU, will be incinerated in a Government incineration facility in Kigali (see RU SOP for Investigational Medicinal Product Destruction).

11.4 Adherence assessments

Adherence to the study interventions will be assessed at the Day 7, Month 1 and Month 2 visits by structured interviewer-administered questionnaire, review of a diary card that the participant will be asked to complete in between study visits, review of returned used packaging and unused product, and by asking the participant to complete a self-rating adherence scale. These different sources of data will be triangulated to arrive at an overall level of adherence for each participant over time.

12. PHARMACOVIGILANCE

12.1 Definition of adverse events

An adverse event (AE) is any untoward medical occurrence, and a social harm any untoward social occurrence, in a study participant during study participation, whether or not there is a causal relationship with the intervention that the participant received.
An unexpected AE is an AE, the nature or severity of which is not consistent with the applicable product information (Summary of Product Characteristics or Investigator's Brochure). The final determination of whether an AE is unexpected will be made by the Chief Investigator, but the Principal Investigator should also be aware of all applicable product information.

12.2 Definition of serious adverse events

A serious adverse event (SAE) is an AE that results in death, is life-threatening, requires or prolongs hospitalisation, results in a congenital anomaly/birth defect, or results in persistent or significant incapacity or disability.

An suspected unexpected serious adverse event (SUSAR) is an SAE, the nature or severity of which is not consistent with the applicable product information (Summary of Product Characteristics or Investigator's Brochure). The final determination of whether an AE is unexpected will be made by the Chief Investigator, but the Principal Investigator should also be aware of all applicable product information.

12.3 Assessing AE/SAE severity and relationship to study products

The Principal Investigator is responsible for assessing the severity of AEs/SAEs occurring on study. The following criteria will be used:

- Mild: Transient or mild discomfort (<48 hours), no medical intervention/therapy required;
- Moderate: Mild to moderate limitation in activity, some assistance may be needed, no or minimal medical intervention/therapy required;
- Severe: Marked limitation in activity, some assistance usually required, medical Intervention or therapy required.

The Principal Investigator is responsible for determining the relationship of all AEs/SAEs with study product use based on the following criteria:

- Not Related: There is no temporal or causal relationship to the study product administration, and the AE/SAE is clearly explained by another cause (concurrent disease, concomitant medication, etc.);
- Probably Not Related (Unlikely): There is a temporal relationship to study product administration, but there is no reasonable causal relationship, and the AE/SAE is more likely explained by another cause (concurrent disease, concomitant medication, etc.);
- Possibly Related: The AE/SAE can be explained by another cause but the possibility of a relationship with the study product cannot be ruled out.
- Probably Related: There is a temporal and causal relationship between the study product and the AE/SAE, and the AE/SAE is more likely explained by the study product than by another cause;
- Definitely Related: There is a temporal and causal relationship between the study product and the AE/SAE, and the AE/SAE responds to withdrawal of the study product (and recurs with repeat administration of the study product).

12.4 Reporting of AEs/SAEs

All SAEs, whether or not deemed expected or study product-related, must be reported by the Principal Investigator to the Chief Investigator within 24 hours using a standardised SAE Notification Form by email (j.vandewijgert@liv.ac.uk). The SAE Notification Form should be completed with all available information at the time of reporting. The Principal Investigator is required to write a second detailed written report after SAE follow-up has been completed. SAE follow-up will be considered completed when the participant returns to her usual health or until the Principal Investigator does not expect further improvement or worsening of the event.

At study visits, some AEs/SAEs will be reported spontaneously when asking if any medical or social problems occurred (unsolicited) and some will be reported only after specific questioning (solicited). Participants will be encouraged to contact study staff in between study visits to report SAEs, AEs that they suspect are product-related, and new episodes of STIs, BV or vaginal candidiasis. They will be allowed to contact study staff for any other AEs in between study visits as well. Treatment, counselling, or referral for SAEs, AEs or social harms that are judged possibly/probably/definitely related to study participation will be provided by RU staff, to the extent possible, at no cost to the

participant. All SAEs and AEs will be followed up until resolution or until the Principal Investigator does not expect further improvement or worsening of the event. Should a participant become pregnant during the study, she will be followed up by the study team until delivery, and the pregnancy outcome will be documented. Any pregnancy outcome that meets the criteria for SAE reporting (e.g. congenital anomalies) will be reported as an SAE.
SAEs and AEs will be reported to the ethics committees listed in section 10.1 in accordance with their guidelines.

13. OTHER

13.1 Funding

This study is funded by the UK Medical Research Council (MRC). The main funding contract is between the UK MRC and the UoL IGH (grant number MR/M017443/1) and by subcontract between UoL IGH and RU.

13.2 Study management and oversight

The trial sponsor is the University of Liverpool (UoL) and this protocol and other trial documentation was therefore reviewed and approved by the UoL Sponsorship Committee. Overall trial 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. VMB testing will take place at the UoL Centre for Genomics Research.

Trial implementation in Rwanda will be managed by the RU team, led by the Principal Investigator Stephen Agaba. He will work closely with the RU community outreach, clinical, laboratory, data management, and social science team leaders. All clinical and social science procedures, as well as diagnostic laboratory testing, will take place at RU according to RU SOPs and using RU source documents.

A Trial Steering Committee was established, but a Data Safety Monitoring Board is not deemed necessary. The reason for this is that all products to be used in this trial are available on the market in Europe, the USA or Rwanda, and are considered low risk. The Trial Steering Committee will consist of the Chief Investigator, Principal Investigator, and external members listed in section 1: Protocol Team Roster. The Committee will be coordinated by the Chief Investigator (Prof. Janneke van de Wijgert) and will meet once prior to study initiation, once during data collection, and once after completion of data collection. The Committee will also be asked to provide input in study documents and procedures via email, and to review each SAE as they become available. In case of major safety concerns, the Trial Steering Committee Members may request the Chief Investigator to halt recruitment of the trial and/or to organize a formal data review of all available safety data. The responsibilities of and procedures related to the Trial Steering Committee are described in detail in its terms of reference.

13.3 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.

13.4 GC(L)P monitoring

This study will be implemented in accordance with the ICH-GCP and Good Clinical Laboratory Practice (GCLP) guidelines (1). We will implement the following monitoring procedures (these are described in detail in the study-specific monitoring plan):

- The Chief Investigator will travel to Kigali to conduct the study training and study initiation visit.
- During data collection, 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). In addition, RU data staff will conduct monthly 100% source data verification.
- A local independent monitor (Jeanine Nyinawabega) will conduct three monitoring visits during the study: approximately 6 weeks, 4.5 months and 8.5 months after the start of data collection.
- The Chief Investigator will travel to Kigali again to conduct the study close-out visit.
- The independent monitor may conduct additional monitoring visits if required. This will be decided by the Chief Investigator based on findings during previous monitoring visits.

13.5 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 Biomedical Centre 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.

13.6 Dissemination of results

Study results will be disseminated as follows:

- To the study participants and community mobilisers via a report-back meeting at RU
- To the research and public health community in Rwanda via presentations by RU or UoL staff at national meetings
- To the international research and public health community via presentations by RU or UoL staff at UoL and international meetings, publications in scientific journals, and publications on the UoL and RU websites.

Publications and authorships on publications will be governed by a separate data and specimen sharing agreement. The agreement will be written by the Chief Investigator with input from all other investigators and external advisors.

13.7 Archiving

Study records will be kept at RU according to the RU SOP for Archiving. RU will retain all study records for at least three years after completion of the last study visit. Documentation may not be destroyed without written approval from the Chief Investigator.

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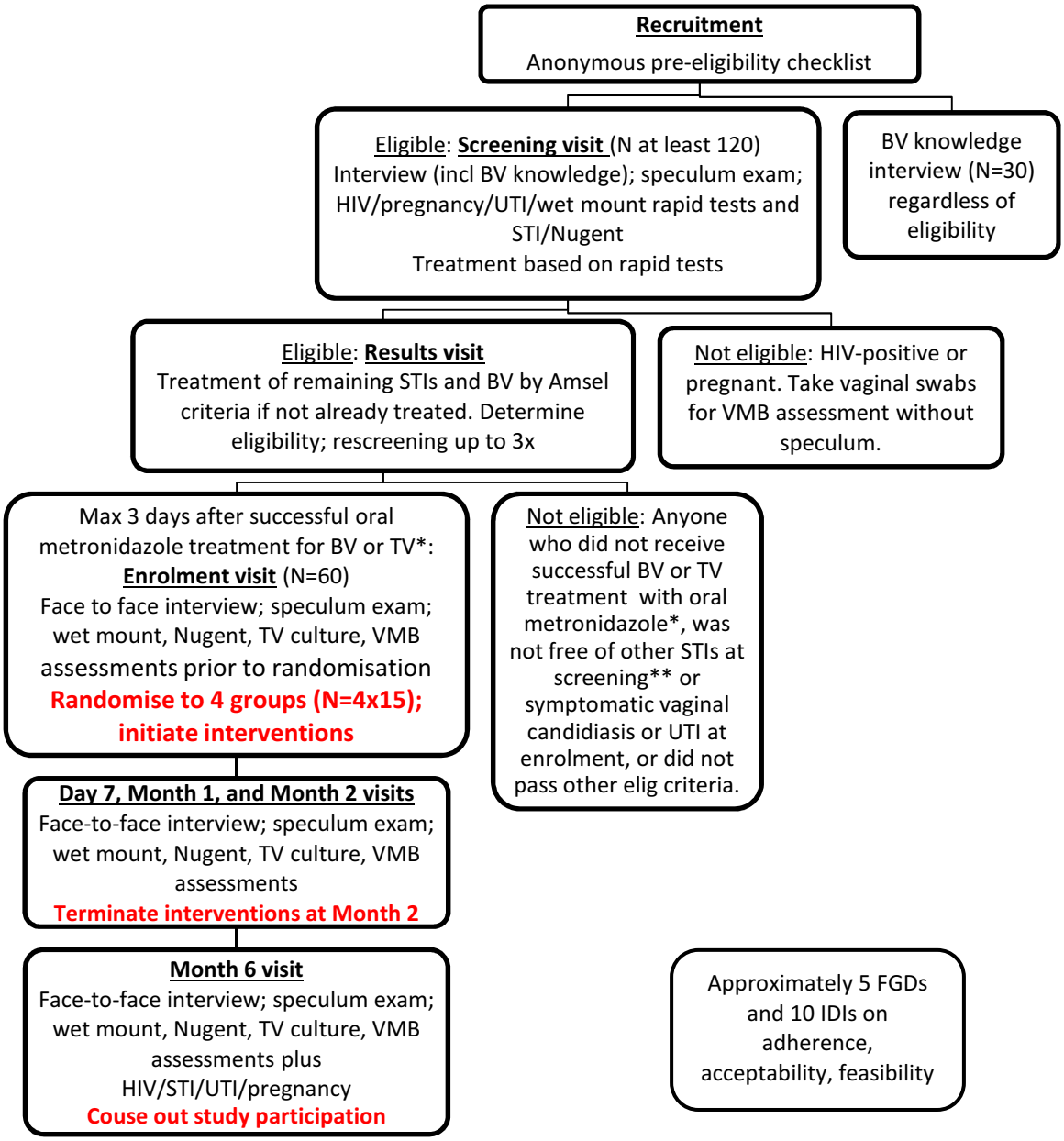
15. APPENDICES

15.1 Visit flow chart



*Successful BV or TV treatment is defined as oral metronidazole treatment for 7 days, after which there was no BV by Amsel criteria and no TV on wet mount.
**Women may be enrolled when their NG and CT results are not yet known. However, a new participant will be randomised for each woman whose screening sample comes back positive for NG and/or CT after having been randomised. The NG/CT-positive women will be allowed to complete the study but their data may be removed from some analyses.

15.2 Schedule of assessments

Procedures in clinic during visits	Screening/ results	Enrolment	D7, M1, M2	M6	FGD/IDI
Informed consent	X	X			X
Contact information	X	X	X	X	X
Assign PIN	X				
Eligibility checklist	X	X			
Randomisation: initiate interventions		X			
Cease interventions			X (M2)		
Face-to-face interview, quantitative	X	X	X	X	
Discussion/interview, qualitative					X
Assess AEs and social harms		X	X	X	
HIV pre- and post-test counselling	X			X	
HIV/STI/BV risk reduction counselling	X	X	X	X	
Blood collection (10 ml)	X			X	
Urine collection	X	X		X	
Rapid HIV testing (whole blood)[1]	X			X	
Rapid pregnancy testing (hCG urine)	X	X	[X]	X	
Rapid UTI testing (urinalysis dipstick)	X	X	[X]	X	
Speculum exam: visual inspection	X[2]	X	X	X	
Speculum exam: vaginal pH + wet mount (TV, candida, BV by Amsel[2])	X[2,3]	X[3]	X[3]	X[3]	
Speculum exam: vaginal swabs	X	X	X	X	
Speculum exam: endocervical swabs	X[2]	X	[X]	X	
Speculum exam: CVL	X[2]	X	X	X	
Provide results and treatment if appl[1]	X	X	X	X	
Laboratory procedures after visits					
Blood: syphilis and HSV-2 serology	X			X[4]	
Endocervical swabs: NG/CT PCR	X	[X][5]	[X][5]	X	
Vaginal swabs: TV InPouch culture	X	X	X	X	
Vaginal swabs: Nugent scoring	X	X	X	X	
Vaginal swabs: store for VMB MiSeq	X	X	X	X	
Laboratory procedures if funding permits:					
Endocervical swab: store for HPV	X			X	
Vaginal swabs: store for additional VMB assessments (e.g. qPCR, biofilm)	X	X	X	X	
CVL: store for additional VMB assessments	X	X	X	X	
Urine: store for urine microbiome		X		X	

AE=adverse event; BV=bacterial vaginosis; CT=*Chlamydia trachomatis*; CVL=cervicovaginal lavage; D7=Day 7 visit; FGD=focus group discussion; HPV=human papillomavirus; HSV-2=herpes simplex type 2; IDI=in depth interview; M1/2/6=Month 1/2/6 visit; NG=*Neisseria gonorrhoeae*; PIN=participant identification number; qPCR=quantitative PCR; STI=sexually transmitted infection; UTI=urinary tract infection; TV=*Trichomonas vaginalis*; VMB=vaginal microbiome.

1. By the algorithm recommended in the most recent Rwandan HIV voluntary counselling and testing guidelines
2. HIV-positive and pregnant women will not undergo a speculum exam unless clinically indicated but they will be asked to donate vaginal swabs and urine (left-over from the pregnancy and UTI testing) for VMB and urine microbiome assessments.
3. BV by Amsel criteria will always be treated during the screening process but will only be treated when symptomatic during follow=up. Candida on wet mount will also only be treated when symptomatic. TV on wet mount will always be treated.
4. For HSV-2 only when negative at baseline.
5. Endocervical swabs will be tested for NG/CT by PCR at screening and M6, but may also be tested at enrolment and/or D7/M1/M2 if deemed necessary for clinical reasons or to accurately interpret the VMB findings.

15.3 Package inserts for metronidazole oral tablets, metronidazole vaginal gel, Ecologic Femi, and Gynophilus

Metronidazole oral tablets

EFLARON®
Metronidazole BP tablets
Metronidazole benzoate BP suspensions

Composition:
Each tablet contains 200 mg, 250mg, 400mg, or 500mg of Metronidazole BP
Each 5ml spoonful of suspension contains 125mg or 200mg Metronidazole (as Benzoate) BP

Pharmacology:
Metronidazole is a 5-nitroimidazole derivative with activity against anaerobic bacteria and protozoa. It also has a radiosensitising effect on hypoxic tumour cells. Its mechanism of action is thought to involve interference with DNA by a metabolite in which the nitro group of metronidazole has been reduced. Metronidazole is active against several protozoa including *Balantidium coli, Blastocystis hominis, Entamoeba histolytica, Giardia intestinalis* (*Giardia lamblia*), and *Trichomonas vaginalis*. Most obligate anaerobic bacteria, including *Bacteroides* and *Clostridium* spp., are sensitive *in vitro* to metronidazole. It is bactericidal. It also has activity against the facultative anaerobes *Gardnerella vaginalis* and *Helicobacter pylori* and against some spirochaetes. Metronidazole has well-established bactericidal activity against obligate anaerobic bacteria *in vitro*, including the Gram-negative organisms *Bacteroides fragilis* and other *Bacteroides* spp., *Fusobacterium* spp., and *Veillonella* spp., and the Gram-positive organisms *Clostridium difficile, Cl. perfringens*, and other *Clostridium* spp., *Eubacterium* spp., *Peptococcus* spp., and *Peptostreptococcus* spp.; *Propionibacterium* and *Actinomyces* spp. are often resistant. It also has activity against the facultative anaerobe *Gardnerella vaginalis*, although its bactericidal effect is reported to be much slower than against obligate anaerobes, against some strains of *Campylobacter* spp. including *C. fetus* subsp. *jejuni*, and against *Helicobacter pylori*. The oxidative metabolites of metronidazole also have antibacterial activity; the hydroxy metabolite has been reported to be consistently more active than metronidazole against strains of *G. vaginalis*.

Pharmacokinetics:
Metronidazole is readily and almost completely absorbed after oral doses. Peak plasma concentrations of about 6 and 12 micrograms/mL are achieved, usually within 1 to 2 hours, after single doses of 250 and 500 mg respectively. Some accumulation occurs and consequently there are higher concentrations when multiple doses are given. Absorption may be delayed, but is not reduced overall by food. Metronidazole benzoate given by mouth is hydrolysed in the gastrointestinal tract to release metronidazole, which in turn is then absorbed. Peak steady-state plasma concentrations of about 25 micrograms/mL with trough concentrations of about 18 micrograms/mL have been reported in patients given an intravenous loading dose of 15 mg/kg followed by 7.5 mg/kg every 6 hours.Metronidazole is widely distributed. It appears in most body tissues and fluids including bile, bone, breast milk, cerebral abscesses; CSF, liver and liver abscesses, saliva, seminal fluid, and vaginal secretions, and achieves concentrations similar to those in plasma. It also crosses the placenta and rapidly enters the fetal circulation. No more than 20% is bound to plasma proteins. Metronidazole is metabolised in the liver by side-chain oxidation and glucuronide formation. The principal oxidative metabolites are 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (the hydroxy metabolite), which has antibacterial activity and is detected in plasma and urine, and 2-methyl-5-nitroimidazole-1-acetic acid (the acid metabolite), which has virtually no antibacterial activity and is often not detected in plasma, but is excreted in urine. Small amounts of reduced metabolites, acetamide and *N*-(2-hydroxyethyl) oxamic acid (HOA), have also been detected in urine and are probably formed by the intestinal flora. The elimination half-life of metronidazole is about 8 hours; that of the hydroxy metabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and in patients with severe hepatic impairment; that of the hydroxy metabolite is prolonged in patients with substantial renal impairment. The majority of a dose of metronidazole is excreted in the urine, mainly as metabolites; a small amount appears in the faeces.

Indications:
Anaerobic infections: gynaecological and intra-abdominal infections, infections of the CNS, pulmonary infections, septicaemia, endocarditis, infections caused by susceptible anaerobic bacteria: Bacteroidec species, including *B. fragilis* group (*B. distosonis, B. ovatus, B. thetaiotaomicron, B. vulgatus*), Clostridium species, Eubacterium species, Peptococcus species, Peptostreptococcus species. Ulcerative gingivitis Infections caused by Trichomonas in both sexes. Amoebiasis Lambliasis and *Helicobacter pylori* eradication.

Dosage and Administration:
Anaerobic infections
Treatment of anaerobic infections (usually treated for 7 days and for 10 days in antibiotic – associated colitis), by mouth either 800mg initially then 400mg every 8 hours or 500mg every 8 hours.
Children: 7.5mg / kg every 8 hours.
Preventive treatment: adults and children more than 12 years old: (100ml) administered in slow intravenous drip infusion immediately before, or during operation; the same dose is repeated every 8 hours until oral treatment is possible (200mg to 400mg) 3 times daily. The treatment (intravenous and oral together) should not last more than a week.
Children less than 12 years old: 7.5mg/kg body weight (=1.5ml/kg) administered in slow intravenous drip infusion following the same schedule as in adults.
Orally, a dosage of 3.7 to 7.5 mg/kg body weight is administered 3 times daily. The complete treatment lasts 7 days.
Trichomoniasis
Both partners should be treated simultaneously. Metronidazole is given by mouth either as a single 2-g dose, as a 2-day course of 800 mg in the morning and 1.2 g in the evening, or as a 7-day course of 600 mg to 1 g daily in two or three divided doses. If treatment needs to be repeated, an interval of 4 to 6 weeks between courses has been recommended.
Children with trichomoniasis may be given a 7-day course of metronidazole by mouth as follows: 1 to 3 years, 50 mg three times daily; 3 to 7 years, 100 mg twice daily, and 7 to 10 years, 100 mg three times daily. An alternative children's dose is 15 mg/kg daily in divided doses for 7 days.
Amoebiasis
Metronidazole is given in doses of 400 to 800 mg three times daily by mouth for 5 to 10 days. Children aged 1 to 3 years may be given one-quarter, those aged 3 to 7 years one-third, and those aged 7 to 10 years one-half the total adult daily dose; alternatively 35 to 50 mg/kg daily in divided doses has been used. An alternative adult dose is 1.5 to 2.5 g as a single daily dose for 2 or 3 days.
Lambliasis:
Adults: 800mg daily, divided into two doses for a period of 5 days.
Children: 35mg to 50mg/kg body weight divided into two doses for a period of 5 days
Leg ulcers and pressure sores
400mg every 8 hours for 7 days by mouth.
Bacterial vaginosis
400 – 500mg twice daily for 5 - 7 days or 2g as a single dose by mouth.
Pelvic inflammatory disease
400 mg twice daily for 14 days
Acute ulcerative gingivitis
Adults: 200 - 250mg daily every 8 hours for 3 days
Children 1 – 3 years: 50mg daily every 8 hours for 3 days, 3 – 7 years 100mg every 12 hours and 7-10 years 100mg every 8 hours.
Acute oral infections
Adults: 200mg daily every 8 hours for 3 - 7 days
Children 1 – 3 years: 50mg daily every 8 hours for 3 - 7 days, 3 – 7 years 100mg every 12 hours and 7-10 years 100mg every 8 hours.
Surgical prophylaxis
Adults: 400 - 500mg daily every 2 hours before surgery; upto 3 further doses of 400 - 500 mg may be given every 8 hours for high – risk procedures.
Children : 7.5mg / kg 2 hours before surgery; upto 3 further doses of 7.5 mg / kg may be given every 8 hours for high – risk procedures

Side effects:
Nausea, gastrointestinal pain, diarrhea, metallic taste may occur. With protracted intravenous use, transient neutropenia as well as peripheral neuropathy, headache, sleepiness, dizzinoss, ataxia and par aesthesia may occur.

Contraindications:
Hypersensitivity to metronidazole or other nitro- imidazole derivatives.
The first trimester of pregnancy.
If CNS disorders occur (ataxia, paraesthesia), treatment should be discontinued immediately. Concomitant administration of disulfiram is contraindicated too.

Caution:
When warfarin or other anticoagulants are administered concomitantly, the dose should be adequately reduced. Patients should abstain from alcoholic beverages during treatment. Metronidazole is excreted with human milk and penetrates the placental barrier. The use of metronidazole during pregnancy and lactation is not recommended.

Presentation:
HDPE jars with 1000 tablets of 200mg / 250mg
HDPE jars with 500 tablets of 400mg/500mg.
Blister pack of 10 X 10's tablets
60ml and 100ml amber coloured bottles

Storage conditions:
Store below 30°C. Protect from light.
Keep all medicines out of reach of children.

Manufactured by:
DAWA Limited, Plot No. 7879/8, Baba Dogo Road, Ruaraka
P. O. Box 16633 – 00620, Nairobi, Kenya,

Ref: Lf /DL/Eflaron /02
Date of issue: July 2013

Metronidazole vaginal gel

PATIENT INFORMATION

Metronidazole (metro-NI-da-zole) vaginal gel 1.3%

For intravaginal use only. Do not use in the eyes, mouth or skin.

What is Metronidazole vaginal gel 1.3%?

Metronidazole vaginal gel 1.3% is used to treat bacterial vaginosis in women who are not pregnant.

Who should not use Metronidazole vaginal gel 1.3%?

Do not use Metronidazole vaginal gel 1.3% if you:

- are allergic to metronidazole, parabens, nitroimidazole derivatives, or any of the ingredients in Metronidazole vaginal gel 1.3%. See the end of this leaflet for a complete list of ingredients in Metronidazole vaginal gel 1.3%.
- take or have taken a medicine called Antabuse (disulfiram) in the last 2 weeks.
- drink alcohol. Do not drink alcohol while you use Metronidazole vaginal gel 1.3% and for at least 24 hours after you stop using it. It can increase your chances of getting serious side effects.

Before you use Metronidazole vaginal gel 1.3%, tell your healthcare provider about all your medical conditions, including if you:

- are pregnant or plan to become pregnant. It is not known if Metronidazole vaginal gel 1.3% will harm your unborn baby.
- are breastfeeding or plan to breastfeed. Metronidazole vaginal gel 1.3% passes into breast milk and may harm your baby. You and your healthcare provider should decide if you will use Metronidazole vaginal gel 1.3% or breastfeed.

Tell your healthcare provider all about the medicines you take, including prescription and over-the-counter medicines, vitamins and herbal supplements.

How should I use Metronidazole vaginal gel 1.3%?

- See the "Instructions for Use" at the end of this Patient Information leaflet for detailed instructions about how to use Metronidazole vaginal gel 1.3%
- Use Metronidazole vaginal gel 1.3% exactly as your healthcare provider tells you to
- Metronidazole vaginal gel 1.3% is used one time at bedtime
- If you get Metronidazole vaginal gel 1.3% in your eyes, rinse your eyes with cool tap water and call your healthcare provider

What should I avoid while using Metronidazole vaginal gel 1.3%?

Do not have vaginal intercourse or use other vaginal products (such as tampons or douches).

What are the possible side effects of Metronidazole vaginal gel 1.3%?

The most common side effects of Metronidazole vaginal gel 1.3% include yeast infections, headache, vaginal itching, nausea, diarrhea, and pain with menstrual cycle.

Tell your healthcare provider if you have any side effect that bothers you or that does not go away. These are not all the side effects of Metronidazole vaginal gel 1.3%. For more information, ask your healthcare provider or pharmacist. Call your healthcare provider for medical advice about side effects. You may report side effects to the FDA at 1-800-FDA-1088.

General information about Metronidazole vaginal gel 1.3%

Medicines are sometimes prescribed for purposes other than those listed in the Patient Information leaflet. Do not use Metronidazole vaginal gel 1.3% for a condition for which it was not prescribed. Do not give Metronidazole vaginal gel 1.3% to other people, even if they have the same symptoms you have. It may harm them.

This Patient Information leaflet summarizes the most important information about Metronidazole vaginal gel 1.3%. If you would like more information, talk with your doctor. You can also ask your pharmacists or doctor for information about Metronidazole vaginal gel 1.3% that is written for health professionals.

For more information call: Valeant Pharmaceuticals North America LLC at 1-800-321-4576

What are the ingredients in Metronidazole vaginal gel 1.3%?

Active ingredients: metronidazole

Inactive ingredients: polyethylene glycol 400, propylene glycol, benzyl alcohol, methylparaben, propylparaben, purified water, and polycarbophil AA-1

How should I store Metronidazole vaginal gel 1.3%?

- Store Metronidazole vaginal gel 1.3% at room temperature, 59°-86°F (15°-30°C).

Keep Metronidazole vaginal gel 1.3% and all medicines out of the reach of children.

This Patient Information and Instructions for Use have been approved by the US Food and Drug Administration.

Rx only

Manufactured for:
Valeant Pharmaceuticals North America LLC
Bridgewater, NJ 08807

Manufactured by:
DPT Laboratories, Ltd.
San Antonio, TX 78215

U.S. Patent 7,893,097

03/2014

Ecologic Femi

Ecologic® FEMI+ 928

Product description:

Light beige powder with good flow-properties. Reasonably dissolvable in water.

Composition:

Cellulose, Bacterial strains, Magnesium Stearate and Lactoferrin

Bacterial strains:

Bifidobacterium bifidum W28
Lactobacillus acidophilus W70
Lactobacillus brevis W63
Lactobacillus helveticus W74
Lactobacillus plantarum W21
Lactobacillus salivarius W24

Packaging:

Laminated aluminium bags (PET/ALU/PE) in cardboard boxes (20kg).

Shelf-life:

Target total cell count lactic acid bacteria two years after production, stored in original closed packaging below 25°C:	≥ 3,0 x 10^9 cfu/g

Physical analysis:

Moisture	< 5 %

Microbiological analysis:

Salmonella spp.	absent in 25 gram
Escherichia coli	absent in 1 gram
Staphylococcus aureus	absent in 1 gram
Bacillus cereus	< 500 cfu/gram
Moulds & Yeasts	< 500 cfu/gram

Gynophilus

GynOphilus® LP

Probiotic *Lactobacillus casei rhamnosus Döderlein*

WHAT CAUSES VAGINAL FLORA IMBALANCE?

Vaginal flora imbalance has several identifiable causes: recent antibiotic treatment, tampon use, very heavy periods, excessive cleansing, repeated use of antifungal agents. Even if the exact cause is not always known, vaginal flora balance absolutely must be protected. It is an essential factor in limiting the occurrence of vaginal infections.

WHAT ARE THE SYMPTOMS OF VAGINAL FLORA IMBALANCE?

Itching, unpleasant odours, heavier discharge, perineal discomfort, irritation…

HOW CAN BALANCE BE RESTORED TO THE VAGINAL FLORA?

Thanks to its natural ingredients, Gynophilus® LP naturally restores balance to the vaginal flora.
Its unique composition (Lcr Regenerans®) combines probiotics (*Lactobacillus casei rhamnosus Döderlein*) that are naturally present in the vagina, with prebiotics (nutritive elements) conferring it triple action:

- It maintains vaginal pH thanks to the lactic acid produced by the lactobacilli
- It creates a protective barrier against infectious agents
- It creates an environment favourable to healthy vaginal flora thanks to the prebiotics

One Gynophilus® LP tablet continually releases Lcr Regenerans® over four days.

WHAT ARE THE ADVANTAGES OF CONTROLLED-RELEASE?

Gynophilus® LP is the first controlled-release vaginal probiotic. It's unique formulation makes vaginal probiotics easier to use:

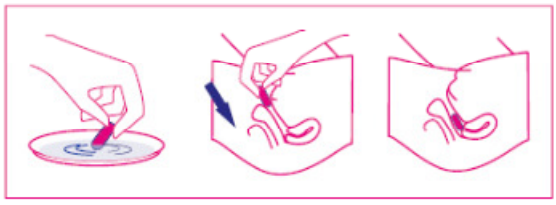
- One tablet contains the quantity of *lactobacilli* required for 4 days
- Controlled-release reduces usually frequent discharge caused by administration of vaginal products.

The tablet disintegrates inside the vagina, forming a gel which continually releases the prebiotics and probiotics, thus providing continuous nutrients and lactobacilli inside the vagina, favourable to their proper implantation and regeneration of the flora.

HOW DO I USE GYNOPHILUS® LP?

Wet the tablet in water to make insertion easier.

Insert a tablet deep in the vagina at night when going to bed.



GynOphilus® LP

Probiotic *Lactobacillus casei rhamnosus Döderlein*

GYNOPHILUS® LP INSTRUCTIONS FOR USE:

1 tablet every four days:
- to regenerate the vaginal flora: 2 tablets treatment
- to prevent relapse: 6 tablets treatment

Gynophilus® LP can be used:
- after a local antifungal or antibiotic treatment
- at the same time as an oral treatment (generally antibiotic)

DURING TREATMENT:
- Discharge may occur a few hours after having inserted the tablet (it is advisable to wear a liner).
- Sexual intercourse possible 8 hours after insertion of the tablet.

PRACTICAL TIPS:
- Wash your hands carefully before and after administration of the product.
- Use soap with neutral or alkaline pH for your intimate hygiene routine.
- Use your own bathroom linen (flannel, towel).
- Preferably wear cotton underwear.
- Avoid vaginal douching.

DO NOT SWALLOW.
KEEP OUT OF THE REACH OF CHILDREN.

PACK SIZE:
Aluminium blister pack containing 2 tablets.

COMPOSITION:
Each Gynophilus® LP tablets contains:
- 876,9 mg of Lcr Regenerans®

STORAGE:


Store below 25 °C.


Do not use after the expiry date on the pack

Manufacturer:
Probionov
Rue des Frères Lumière
15130 Arpajon-sur-Cère - FRANCE

Distributor:
XXXXXXXXX
XXXXXXXXXXXX
XXXXXXXXXXXX

CE 0537

probionov
Treat Different

Distributor logo

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