ITM Clinical Trial ITM201801 – NCT03881007

A double-blind single center, crossover, randomized controlled trial of antibacterial vs. placebo mouthwash to reduce the incidence of gonorrhea/chlamydia/syphilis in MSM taking HIV pre-exposure prophylaxis (PrEP)

Study Acronym: PReGo

Initial Protocol
Version 2.1, 08-Jan-2020

Sponsor: Institute of Tropical Medicine
Nationalestraat 155
B-2000 Antwerpen - Belgium

Coordinating Investigator: Dr. Chris Kenyon

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Study Title:	A double-blind single center, crossover, randomized controlled trial of antibacterial vs. placebo mouthwash to reduce the incidence of gonorrhea/chlamydia/syphilis in MSM taking HIV pre-exposure prophylaxis (PrEP)
Study Acronym:	PReGo
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Sponsor study team

Institute of Tropical Medicine

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STATEMENT OF COMPLIANCE

By signing this protocol, the Investigator(s) acknowledge(s) and agree(s):

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the Declaration of Helsinki, Good Clinical [Laboratory] Practice (GC[L]P), the EU General Data Protection Regulation (GDPR), the ESF/ALLEA Code of Conduct for Research Integrity, and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

The Sponsor of this study – the Institute of Tropical Medicine in Antwerp, Belgium (ITM) – can at any time have access to the source documents from which Case Report Form information may have been generated and will be permitted to perform trial-related monitoring and audits. All study material will be maintained according to regulatory requirements and until the Sponsor advises that retention is no longer necessary.

COORDINATING INVESTIGATOR/PRINCIPAL INVESTIGATOR:

Title, Name: Dr. Chris Kenyon Date: _____

Signed:

DIRECTOR OF THE INSTITUTE OF TROPICAL MEDICINE:

Title, Name: Dr. Marc-Alain WIDDOWSON Date: _____

Signed:

Signing this document, I commit to carry out the trial in accordance with the protocol, Good Clinical Practice and applicable ethical and regulatory requirements. I also acknowledge the paragraph relevant to study confidentiality and authorize the Institute of Tropical Medicine, Antwerp, Belgium to record my data on a computerized system containing all the data pertinent to the study.

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SYNOPSIS

HYPOTHESIS	LCM compared to placebo mouthwash (taken daily and pre/post sex) is able to reduce the incidence of gonorrhea (Ng)/chlamydia (Ct)/syphilis in higher risk MSM
DESIGN	Double-blind, single center cross-over randomized controlled trial
STUDY SITE & POPULATION	Subjects will be recruited from the existing PrEP cohort at the Institute of Tropical Medicine Antwerp. A total of 320 subjects will be recruited (160 in each arm).
DURATION	Each participant will be enrolled for 6 months (2x 3 months, no wash out period). Total study duration will be 14 months.
OBJECTIVES AND ENDPOINTS	**Primary objective:** Assess if there is a difference in the incidence rate of Ng, Ct and syphilis detected at any site whilst individuals are on LCM vs. placebo. **Endpoint:** Cumulative number of new diagnoses of any combination of Ng, Ct and syphilis in a 3-month period on LCM/placebo.
INCLUSION & EXCLUSION CRITERIA	**Inclusion:** 1. Men aged 18 or more 2. Enrolled in Belgian PrEP program at ITM 3. Has had sex with another man in the previous year 4. Has had a symptomatic or asymptomatic STI (Ct/Ng/syphilis) in the previous 2 years 5. Willing to be enrolled in the cohort for 6 months and attend 3 monthly follow up visits 6. Willing to comply with the mouthwash study schema and willing to ask their casual partners to mouthwash pre- and post-sex 7. Prepared to fill out the online diary once a week 8. Able and willing to provide written informed consent **Exclusion:**

	1. Currently using a mouthwash and unwilling to cease use of this mouthwash 2. Enrolment in another interventional trial 3. Tests HIV positive at screening
SCREENING, RECRUITMENT & RANDOMIZATION	Subjects (identified from the PrEP cohort) will be screened at the ITM. If eligible, they will be enrolled and randomized to group 1 (LCM) or group 2 (placebo). After 3 months, a crossover will occur and subjects will switch to the other intervention.
STUDY DRUG (& COMPARATOR IF APPLICABLE)	Group 1: Listerine® CoolMint (LCM) mouthwash Group 2: Placebo mouthwash Dose: 20mL during 60 seconds Schedule: Once a day + before and after sex
FOLLOW-UP	3-monthly visits (M0, M3, M6)
SAFETY	Safety of the intervention will be evaluated by recording (Serious) Adverse Events. Data will be monitored by means of a sponsor regulated pharmacovigilance system.
STATISTICAL METHODS	The primary hypothesis of this study will be assessed using a mixed effects Poisson regression model with sum of Ng, Ct and syphilis infections occurring on one person as the outcome variable, treatment (placebo or LCM) and visit as independent variables and a random intercept. The primary objective will be addressed through the p-value of the treatment variable in the model.
QUALITATIVE RESEARCH METHODS	To explore the acceptability of moutwash as an HIV/STI prevention method and the experienced and perceived barriers and facilitators for optimal mouthwash adherence semi-structured interviews will be undertaken with a sub-sample of +/- 20 purposively selected participants. Qualitative research data collection and analysis will be guided by a constant comparative approach.

1. INTRODUCTION

1.1 Background

Countries such as Belgium are currently experiencing epidemics of a range of STI in MSM [2,3]. The prevalence of *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoeae* (Ng) and *Mycoplasma genitalium* (*M. genitalium*) in HIV preexposure prophylaxis (PrEP) cohorts in Belgium and elsewhere is typically around 10% [4-6]. Of greater concern is the repeated emergence of antimicrobial resistance (AMR) in MSM over the past three decades [7,8]. This has been most apparent in the case of Ng where AMR has typically emerged in MSM years before heterosexuals [7-9]. Likewise macrolide resistance in *Treponema pallidum (T. pallidum)* in the United States and Australia, first emerged in predominantly MSM populations [10-12]. We have recently found that 100% of *T. pallidum* in Belgium is resistant to macrolides [13]. In the case of *M. genitalium*, the prevalence of macrolide resistance in MSM in one Australian study was also found to be approximately double that of heterosexual men [14]. A number of outbreaks of highly resistant enteric STIs have also occurred in MSM [15-21].

Both *M. genitalium* and Ng have developed resistance to every antibiotic that has been used against them [22,23]. This has led to serious concerns that if we do not address the underlying causes these infections may become untreatable [9,24,25]. The World Health Organization has labeled this risk of AMR in Ng as a 'global threat' [26,27].

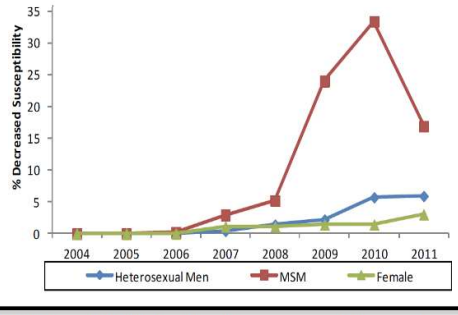
We and others have produced various types of evidence that an important underlying driver of AMR in MSM is a combination of dense sexual networks and excessive antibiotic exposure (the pharmacoecologic theory of AMR) [28-30]. If this is true, then current efforts to reduce STI prevalence via expanded screening and antibiotic therapy in MSM may paradoxically be playing an important role in the promotion of AMR in Ng [29,31].

These considerations provide the motivations for this study where we attempt to reduce the incidence of Ng/Ct/syphilis with a product not known to induce antimicrobial resistance. Listerine Cool Mint® (LCM) has been shown *in vitro* and *in vivo* to be effective against Ng and other STIs [32]. We will assess if daily and pre/post sex LCM mouthwash can reduce the incidence of these STIs in MSM. By reducing the incidence of these infections, we aim to reduce the usage of antimicrobials and thereby reduce the selection pressure for AMR in this key population.

Why does AMR repeatedly emerge in MSM? The pharmacoecologic theory of AMR

It is not known why AMR in Ng has typically emerged in MSM and other core-groups over the past 3 decades (Figure 1) [8]. We have reviewed the available evidence and proposed a pharmacoecologic explanation, postulating this emergence is due to a combination of a dense sexual network plus excessive antibiotic usage within this network (Figure 2) [28-30].

Figure 1: Prevalence of cefixime resistance in MSM and heterosexuals by year, United Kingdom [1]



Step 1: A dense sexual network generates high prevalences of Ng and other STIs

Our group has created a model of Ng transmission in Belgian MSM based on the self-reported behaviours of 3982 Belgian MSM in the European Men who have sex with men Internet Survey [33]. Sixty three percent of these men reported 4 or more partners in the past year and 51% reported partner concurrency [34]. These behavioural parameters resulted in a

dense sexual network and a Ng equilibrium prevalence approximating the 11.1% observed at the screening visit for the PrEP cohort at the Institute of Tropical Medicine [33,35]. The prevalences of Ct and *M. genitalium* at this screening visit were even higher [35]. These prevalences are typical for PrEP and other higher risk MSM cohorts elsewhere with similar risk behaviours [4,5,36].

Step 2: Using antibiotics to drive down Ng/STI prevalence results in a selection pressure for AMR

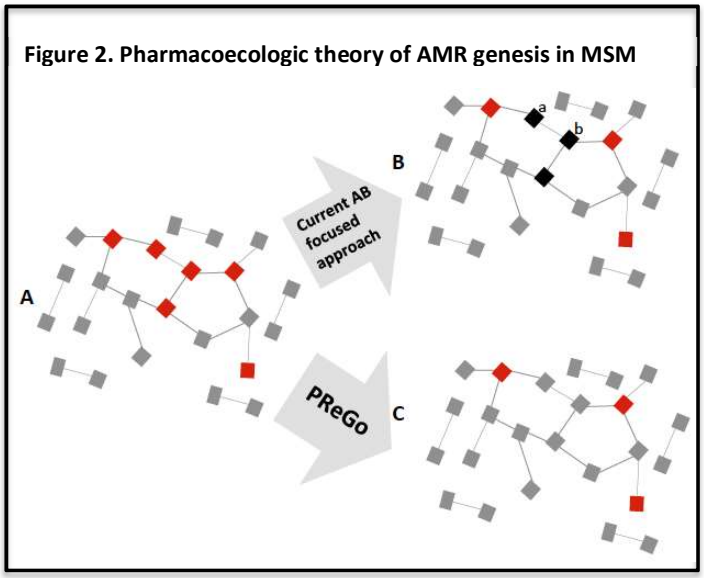
Because of these high STI prevalences, regular screening at 3 monthly intervals (as currently recommended in most PrEP guidelines including Belgium's [37]) will result in a 15 to 20% probability of each individual being exposed to antibiotics at each visit. Each treatment for Ng carriage results in long-term elevations in the prevalence of AMR genes in the microbiomes of treated individuals (up to 4 years for azithromycin) [38]. The available evidence suggests that this strategy of intensive STI screening has a small or no impact on reducing STI prevalence. In a systematic review we conducted on this topic, we found that regular screening for gonorrhoea and chlamydia in MSM (including 3 monthly screening) was not associated with a reduction in the prevalence of these infections [4]. This has also been our experience in the Be-PrEP-ared study (NCT00971230); an HIV pre-exposure prophylaxis demonstration study in Belgian MSM conducted at the Institute of Tropical Medicine (ITM). Despite 3 monthly screening/treatment the prevalence of Ng/Ct/*M. genitalium* have not decreased [35]. The incidence of other STIs such as syphilis, hepatitis C, oncogenic human papilloma viruses and lymphogranuloma venereum has also remained high in our and other PrEP cohorts [5,6,36].

In our model of Ng transmission in Belgian MSM we found that the sexual network was so dense that current levels of screening were only having a small effect on Ng prevalence, mainly because those screened and treated returned to the same dense sexual network which resulted in high probability of reinfection. Increasing screening intensity to 50% coverage annually was found to result in a small decline in NG prevalence but at the cost of a 12-fold increase in antibiotic exposure [33].

Step 3: The combination of network connectivity and antibiotics results in AMR

On the basis of these findings, our pharmacoecologic theory of AMR concludes that contemporary intense screening efforts temporarily reduce the prevalence of Ng with antibiotics. Because the underlying network density is a key determinant of Ng prevalence and this is unaltered, the reduction in Ng prevalence will result in a tendency for Ng to return to its equilibrium Ng prevalence for this degree of network connectivity. One way for Ng to do this is to acquire AMR and this is in turn facilitated by the fact that the screening programme enriches the resistome of the population with the resistance genes that Ng requires to develop AMR [29,30]. These points are illustrated in Figure 2 where a dense sexual network translates into a high equilibrium prevalence of Ng (red squares) in scenario 'A'. Active Ng screening of 50% of this population every 3 months results in a 50% lower Ng prevalence in scenario 'B' (3 months later) but at the expense of an altered resistome (black squares represent 3 individuals with Ng cleared via

Figure 2. Pharmacoecologic theory of AMR genesis in MSM



antibiotics). The unchanged underlying network connectivity then results in a force that pushes Ng back towards its equilibrium prevalence. This places recently cured individuals (such as individuals 'a' and 'b') at high risk of reinfection at a time when their resistomes are enriched with resistance genes. Early reinfecting Ng are able to take up these resistance genes via transformation. Extensive antimicrobial exposure in this setting could also lead to AMR via other mechanisms such as chromosomal mutations. The combination of the high network connectivity and antimicrobial exposure has thereby led to antimicrobial resistance. The PReGO Study design (scenario 'C') aims to reduce the prevalence of Ng with a mouthwash and thus reduce the selection pressure for antimicrobial resistance (Uninfected individuals: grey squares; edges between squares represent sexual relationships). Similar logic would apply to other bacterial STIs [30].

The importance of oropharyngeal gonorrhea in the genesis of antimicrobial resistance

If a combination of high network connectivity and excess antibiotics are responsible for AMR then either or both of these would need to be reduced to decrease the risk of AMR. Initiatives to fragment network connectivity are important but there is little evidence of their efficacy in the current climate [39]. Less emphasis has been placed on reducing antibiotic usage. The PReGo study aims to reduce the prevalence of Ng (and other STIs) in the oropharynx through the use of a mouthwash and thereby reduce antibiotic usage and the probability of AMR emergence in Ng.

The oropharynx is critically important in the emergence of AMR in Ng for a number of reasons: (1) the penetration of numerous antimicrobials into the pharyngeal mucosa is poor leading to a heightened risk of sub-therapeutic levels [40]; (2) the prevalence of Ng is higher here than other body sites [40,41]; (3) this is major habitat for the 10 other *Neisseria* species found in humans [42]. The high prevalence and poor antimicrobial penetration result in a selection pressure for AMR particularly of the abundant commensal *Neisseria* spp. which are then able to transfer resistance conferring genes to incident Ng. Phylogenetic analyses and in-vitro work, for example, have established that Ng acquired resistance to cefixime by taking up DNA conferring cefixime resistance from a commensal pharyngeal Neisseria [24,43].

1.2 Rationale

Main study hypothesis: LCM compared to placebo mouthwash (taken daily and pre/post-sex) is able to reduce the incidence of gonorrhea/chlamydia/syphilis in higher risk MSM

PReGO aims to use a non-antibiotic to reduce the incidence of Ng and other STIs in MSM and thereby reduce overall antibiotic exposure. By reducing pharyngeal Ng it will also reduce the opportunities for Ng to exchange genetic material with commensal *Neisseria* spp. If successful these will reduce the probability of Ng acquiring AMR. This approach differs considerably from certain contemporary guidelines and papers that advocate for increased screening and antibiotic therapy as a way to reduce prevalence of STIs such as Ng in MSM [5,6,37,44,45]. In particular the thinking underpinning PReGo is diametrically at odds with two recent studies that succeeded in reducing the incidence of Ct and syphilis (but not Ng) via doxycycline prophylaxis in PrEP participants [46,47]. Since the publication of these studies, doxycycline is freely available from one of the major online websites providing access to PrEP for HIV (www.iwantprepnow.co.uk)[48]. A major worry with this approach is that it will promote the emergence of AMR in a range of STIs and other bacteria.

LCM-mouthwash as an antibiotic sparing way to reduce STI prevalence

In this study we assess the efficacy of a STI prevention strategy from the pre-antibiotic era. In this era

antiseptics were frequently used to prevent and treat gonorrhea and syphilis [49-51]. Various studies found that the introduction of post exposure urethral antiseptics during the first world war was associated with an up to 20-fold reduction in incidence of symptomatic STIs in the armed forces [49-51]. In one study the introduction of compulsory post exposure urethral antisepsis with calomel/argoyl resulted in a reduction in STIs from 625/1000 to 35/1000/month [52]. In another study similar compulsory prophylaxis resulted in a decline from one in 37 exposures to one in 274 exposures [50]. The efficacy of these interventions resulted in their being made compulsory in the United States armed forces from 1912 to 1939 [51]. There were however no control groups in these interventions and interest in topical antiseptics waned after the discovery of antibiotics [51,53]. This proposal revisits the idea of topical antiseptics based on this historical evidence plus two types of evidence that oral sex plays a critical role in the transmission of Ng [54-56], *T. pallidum* [57-59] and other STIs [56,57,60] in MSM:

1) <u>*Oral sex is common and condoms are rarely used.*</u> HIV prevention efforts have succeeded in increasing the rates of condom usage for anal sex but not for oral sex [61]. Because this is largely due to the unpalatability of condoms in oral sex, surveys have confirmed that around 1% of MSM use [41] and less than 10% of MSM are prepared to use condoms for oral sex [62-64]. As a result, MSM in Belgium and elsewhere are ten times more likely to have condomless oral sex than condomless anal sex with a casual partner [34,54].
2) <u>*Oral contact is an efficient way to transmit certain STIs.*</u> Recent studies have produced evidence that oropharyngeal gonorrhea can be efficiently transmitted by receptive and insertive penile oral sex, tongue kissing, oro-anal and penile-anal sex (via infected saliva used for lubrication) [54,65-67]. Saliva is more infectious than commonly appreciated – 67%/100% of those with pharyngeal Ng detected by PCR have saliva that is culture/PCR positive for Ng [68,69]. The efficiency of transmission plus the frequency of these types of sex, is largely responsible for the fact that the prevalence of Ng is higher in the pharynx than the urethra or rectum in MSM [65,69-71]. Although the underlying pathophysiology of transmission is different, a high proportion of patients with primary/secondary syphilis have positive oral swab PCRs for *T. pallidum* (65% in one large study [72]). In addition case control studies have confirmed that various forms of oral sex acts are independent risk factors for the acquisition of Ng (rectal , urethral and pharyngeal) and syphilis [59,73-75]. As a result the pharynx may be responsible for up to 75% of Ng and 50% of syphilis transmissions in contemporary MSM populations [59,71,74].

These findings provide the rationale for assessing the efficacy of LCM mouthwash in reducing the incidence of bacterial STIs. Mouthwash and gargling pre sex is an established ritual for a small proportion of MSM in Belgium and elsewhere before having sex they perceive as particularly high risk such as commercial sex [32,63,76,77]. Interest in mouthwash is also widespread in MSM. Various qualitative and quantitative studies have found that mouthwashes effective at preventing STIs would be welcomed and widely used by MSM [32,77]. One study, in Australia, for example, asked MSM which of six interventions they would likely adopt to reduce their chance of acquiring gonorrhea. The most popular intervention was daily mouthwash, which 65%/18%/17% reported they were likely/neutral/not likely to carry out [76]. Very few MSM were prepared to use condoms for oral sex or stop having oral sex to reduce their infection risk. Although this study only assessed intent, a different longitudinal study demonstrated a close to 100% adherence amongst MSM to daily Listerine MWG over a 2 week period [77]. Another qualitative study in MSM found considerable interest in the use of a daily mouthwash and a number reported already using Listerine or other mouthwashes pre and post sex [63]. No study has evaluated if LCM or any other mouthwash is able to reduce the incidence of STIs in MSM or any other populations. The PReGo Study would fill this gap.

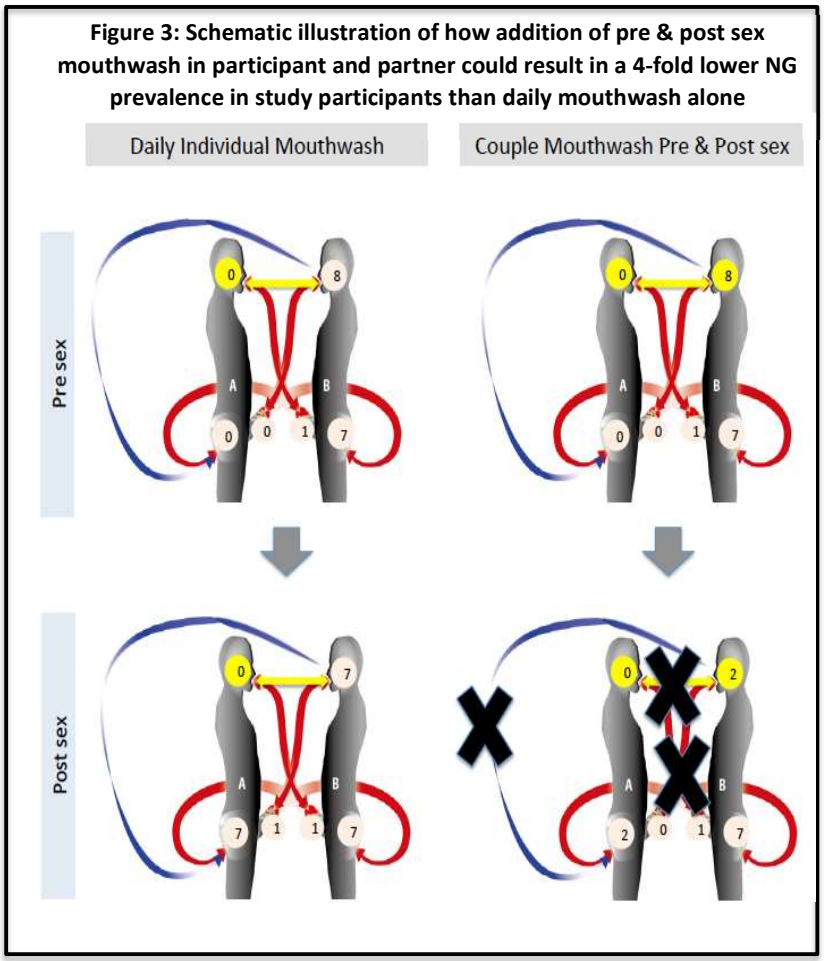
A number of mouthwash products could be used to prevent STIs. Chlorhexidine is regarded by many as the gold standard oral antiseptic [78], and we and others have established that it is rapidly bactericidal

against Ng [79]. However, its major side effect of tooth discoloration makes it unsuitable for long term usage [80]. After an extensive review of the literature we have concluded that LCM mouthwash offers the optimal balance of efficacy and safety. LCM and/or its constituent essential oils have been shown *in vitro* to be bactericidal at concentrations that can be obtained *in vivo* via mouthwashing/gargling to a range of bacterial STIs including Ng [32,81], Ct [82-84], *Haemophilus ducreyi* [85-87] *Mycoplasma* spp. [88]. A recent in-vitro study established that zero colony forming units (CFU)/ml of Ng (from a suspension of 10^8 CFU/ml) were retrieved following 60 seconds contact time with LCM (diluted up to four fold) [32]. As far as syphilis is concerned, LCMs *in vitro* effect on *Treponema pallidum* has not been assessed as this organism cannot be cultured *in vitro*. It has however been found to be highly bactericidal against the closely related oral Treponemes (*Treponema vincentii* and *Treponema denticola* [89]). It is also virucidal to herpes simplex virus 1 and 2 [90,91]. Finally it is cheap, widely available in supermarkets and pharmacies, popular amongst patient groups including MSM [63] and has an excellent safety profile in over 100 years of clinical use [92]. Prophylaxis studies from the preantibiotic period using less bactericidal compounds than LCM found that prophylaxis was equally efficacious against the three then most prevalent STIs – gonorrhoea, chancroid and syphilis [49,93].

LCM has also been shown to work *in vivo* against Ng. A recent randomized controlled trial (RCT) found that a single LCM mouthwash and gargle was able to reduce the culture positivity of pharyngeal Ng by 80% [32]. Our study aims to test if the daily and peri-sexual use of LCM mouthwash is able to reduce the incidence of 3 bacterial STIs and thereby reduce the antimicrobial exposure/risk of AMR development.

The placebo we will use is exactly the same as that used in two previous RCTs except that it does not contain any ethanol or sodium benzoate. We elected to remove the ethanol and sodium benzoate from the placebo as during in vitro testing in our laboratory we found that the placebo including these substances inhibited the growth of Ng. This was not the case with the placebo without these substances.

Figure 3: Schematic illustration of how addition of pre & post sex mouthwash in participant and partner could result in a 4-fold lower NG prevalence in study participants than daily mouthwash alone



The importance of eliminating one's partners' pharyngeal STIs

The study protocol will involve both daily and pre/post sex mouthwashes. A RCT in Australia will test the efficacy of daily mouthwashes on the incidence of pharyngeal Ng [32]. This study design will not prevent the study participants from acquiring these STIs in their pharynx, rectum or urethra during sex (including oropenile, kissing, rimming) with their partner. It will however likely eliminate oral STIs after they have been acquired. The benefit of the added pre/post sex mouthwash is illustrated schematically in Figure 3. In the daily mouthwash scenario (left) only the study participant 'A' mouthwashes (depicted by yellow circle) and thus during sex there is no reduction in transmission of Ng (and other STIs) from his partners ('B') oropharynx to his penis (oral sex), anus (rimming and saliva for penile lubrication) and his mouth (kissing). The numbers inside the circles refer to the Ng prevalences (as percentages) at the 3 Ng niches (urethral, oral and rectal). The scenario commences post a study visit when all Ng would be eradicated in 'A'. After a number of sex episodes with 'B', the prevalence of Ng in the urethra and rectum of 'A' would approximate of that of 'B' (total Ng prevalence 8%). In contrast, if 'A' and 'B' both mouthwash pre and post sex this reduces the prevalence of Ng in 'B's pharynx and this in turn reduces transmission to the urethra, rectum and pharynx of 'A' (total Ng prevalence 2%). Similar considerations would apply to other pharyngeal STIs.

A novel methodology to assess resistogenicity in NG

Our group is in the late stages of constructing an NGmorbidostat. This is an *in vitro* system of generating AMR in Ng by varying the antibiotic concentration relative to Ng growth rate [94]. We are using this system to assess the pharmacoecologic theory of AMR development in MSM (2017 SOFI Pump Prime Project awarded to C Kenyon/T Crucitti).

Although only a small number of studies have evaluated resistance to essential oils, it appears that resistance only occurs in a small number of bacteria and is partial [89,95]. It is unknown if resistance could emerge in Ng or other bacteria, and if so, what the molecular mechanisms would be. To address this critical issue, we will attempt to induce resistance to LCM in both the reference strains of Ng (WHO strain F) and *Neisseria flavescens (N. flavescens)* (NCTC8263T)*,* which is a common pharyngeal commensal and has been shown in transformation and phylogenetic experiments to be readily able to transfer resistant genes to Ng [42]. These experiments will be conducted in the NGmorbidostat by slowly increasing LCM concentrations in response to bacterial growth rates [42,94]. If we find that we can easily induce LCM resistance in Ng or *N. flavescens* this would need to be taken into account in future initiatives to utilize LCM to reduce STI prevalence.

Once we know what mutations (if any) are associated with LCM resistance from this *in vitro* work, we will be able to assess for the presence of these mutations in the Ng and commensal *Neisseria* spp. in the pharyngeal specimens from the LCM and placebo recipients.

2. STUDY OBJECTIVES

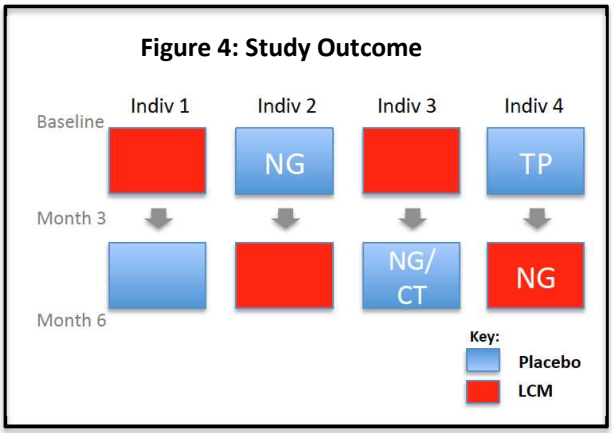
2.1 Primary objective

Assess if there is a difference in the incidence rate of Ng plus Ct plus syphilis detected at any site whilst individuals are on LCM vs. placebo.

Endpoint: The sum of new diagnoses of Ng, CT and syphilis in a 3 month period on LCM/placebo. Each participant can only contribute one diagnosis of Ct and one diagnosis of Ng per visit - regardless of number of sites infected. Thus each participant can contribute up to 3 diagnoses (Ct/Ng/syphilis) at each visit. This is illustrated in Figure 4, which depicts the results for 4 individuals. There is one STI diagnosis in 4 visits whilst on LCM (Ng in individual 4), and 4 STI diagnoses in 4 visits whilst on placebo (2xNg, 1xCt and 1xsyphilis[TP]). Laboratory confirmed Ng, Ct or syphilis infections at the ITM or another center during their inclusion in the study (i.e. between visits), will be self-reported by the participants and will be included in the cumulative new number of Ct/Ng/syphilis diagnoses.

The diagnosis of Ng and Ct will be made via molecular testing and syphilis via RPR and TPA tests according to currently used European case definitions [96].

Figure 4: Study Outcome



2.2 Secondary Objectives

1. Assess if there is a difference in incidence rate of pharyngeal Ng between periods on LCM vs. placebo
   Endpoint: Incidence rate of new pharyngeal Ng cases in each allocation arm (LCM and placebo)
2. Assess if there is a difference in incidence rate of Ct (combined pharyngeal, urethral and rectal) between periods on LCM vs. placebo

   Endpoint: Incidence rate of new Ct cases in each allocation arm (LCM and placebo)

3. Assess if there is a difference in incidence rate of syphilis between periods on LCM vs. placebo
   Endpoint: Incidence rate of new syphilis cases in each allocation arm (LCM and placebo)

4. Describe adherence to (1) daily mouthwash (2) pre/post sex mouthwash
   Endpoints:

  - 1) Daily: Proportion of days in study used mouthwash at least daily
  - 2a) Pre/post sex: Proportion of casual sexual contacts in study when mouthwash used ***pre and post*** sex
  - 2b) Pre sex: Proportion of casual sexual contacts in study when mouthwash used ***pre*** sex

5. Difference in incidence rate of Ng plus Ct plus syphilis between LCM and placebo *after controlling for adherence with daily and pre/post sex mouthwash*
   Endpoint: Incidence rate of Ng, Ct and syphilis between both groups (LCM and placebo) post controlling for adherence to mouthwash
6. Examine whether and how mouthwash is acceptable as a method for preventing STIs among MSM taking PrEP
   Endpoint: Qualitative research analyses
7. Explore experienced and perceived barriers and facilitators for optimal mouthwash adherence among MSM taking PrEP
   Endpoint: Qualitative research analyses
8. Assess if there is a difference in cumulative antibiotic use between periods on LCM vs. placebo
   Endpoint: cumulative number of antibiotics used between both groups (LCM and placebo) for each period
9. Assess if there is a difference in the pharyngeal microbiome/resistome after 3 months on LCM vs. placebo
   Endpoint: Impact of use of LCM vs. placebo on the pharyngeal and rectal microbiome
10. Assess if we can induce resistance to LCM in Ng and *N. flavescens* in the NGmorbidostat and if so which mutations are associated with resistance
    Endpoints:
    - Ng: Time to induction of LCM resistance
    - *N. flavescens:* Time to induction of LCM resistance
11. Assess if LCM-resistance-associated mutations are more prevalent (1) in those receiving LCM vs. placebo (Inter-individual comparison) (2) after 3 months on LCM than before receiving LCM (Intra-individual comparison)
    Endpoint: Prevalence of LCM-resistance-associated mutations in the pharyngeal and rectal microbiome of the LCM group vs placebo-group. Prevalence of LCM-resistance-associated mutations in those receiving LCM after 3 months vs before receiving LCM.
12. Model reduction in probability of development of Ng AMR to ceftriaxone/azithromycin in MSM in Belgium assuming different rates of uptake of LCM mouthwash

3. STUDY DESIGN

3.1 General study design

This is a double-blinded single center, cross-over, randomized controlled trial in MSM taking HIV Pre-exposure prophylaxis (PrEP). A total of 320 Subjects will be recruited and randomized (1:1) to one of the following groups:

1) Listerine ® Coolmint (LCM) mouthwash: daily dose + before and after sex (n= 160)
2) Placebo mouthwash: daily dose + before and after sex (n= 160)

In order to reduce the bias as much as possible, the trial is double-blind, keeping all subjects and the investigators blinded to the IMP.

After 3 months, subjects will switch to the other study group for the next 3 months (until month 6) (see figure 1 study flowchart). At the end of each period (month 3 and month 6), subjects will be screened for STIs and will be asked to complete a CASI (computer assisted self-interview) questionnaire on sexual behavior.

Recruitment will take 12 months. Subjects will be enrolled in the study for 6 months in total. Total study duration will thus be 18 months.

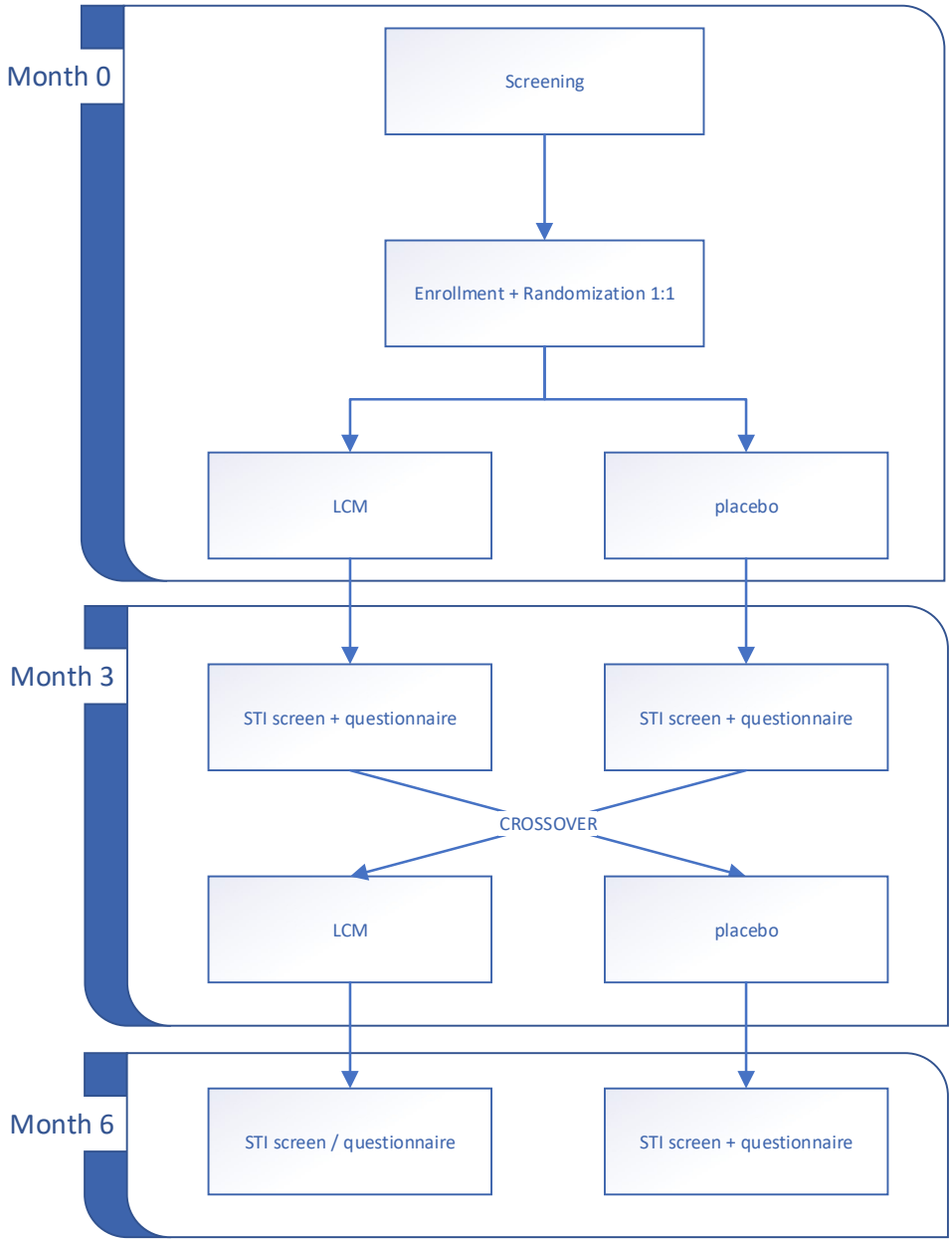


Figure 4: Study flowchart. LCM = Listerine Cool Mint; STI = Sexually Transmitted Infection

3.2 Sub-studies

3.2.1 Assessing acceptability and adherence of mouthwash/gargle

A qualitative study will be embedded within the RCT to address secondary objectives 6 and 7. This will involve semi-structured interviews of a subset of 20 participants or more (depending on data saturation) at 6 months. They will be conducted to gain insights into the acceptability of using mouthwash for prevention purpose, their experiences utilizing the mouthwash and their perspectives towards future adherence of the STI prevention method. For further information see '9. Qualitative research methods'.

3.2.2 Enhanced Resistome Substudy (effect of LCM vs. placebo on the oropharyngeal/rectal microbiome/resistome°

Selection of participants for Enhanced Resistome Substudy analysis
Sixty participants will be selected for the detailed resistome/microbiome analysis substudy. Because previous antibiotic exposure has a well-established effect on the resistome and microbiome this sample will be stratified by history of antimicrobial exposure (30 individuals who report no antibiotics received in the 6 months prior to their enrollment visit and 30 individuals who report being treated with antibiotics at least once in the past 6 months). The sample will also be balanced between those starting the active product and the placebo.
The sampling for these individuals will be conducted as follows:
From the time the study commences enrolling individuals all those enrolled will be included into this substudy if they report either:

- No receipt of antibiotics in the prior 6 months: the first 30 eligible individuals will be included
- Received antibiotics in the previous 6 months: the first 30 eligible individuals will be included

The results of this substudy will be used to determine if further molecular profiling of the pharyngeal and rectal resistome/microbiome will be performed on the stored samples of the rest of the study participants.

4. PARTICIPANTS, POPULATION & SELECTION

4.1 Settings, selection & recruitment

The study will take place within the existing PrEP cohort at the ITM. There are currently 470 MSM PrEP users in 3 monthly follow up at the ITM. The study will be preceded by a community sensitization program that will be conducted in conjunction with the Community Advisory Board.

All eligible subjects will be approached during the 3-monthly visits at the ITM by an ITM study investigator. They will be informed about the study by their routine PrEP physicians and given the opportunity ask questions and decide if they would like to participate. Study posters and information leaflets will be prepared and provided in the ITM HIV/STI clinic waiting rooms. If they agree to participate in the trial, they will be referred to the study doctor who will provide more detailed explanation about the trial. Once the informed consent process has taken place and the ICF has been signed they will be enrolled in the trial.

4.2 Inclusion and exclusion criteria

In order to be eligible, study participants **must meet the following criteria**:

1. Men aged 18 or more
2. Enrolled in Belgian PrEP program at ITM
3. Has had sex with another man in the previous year
4. Has had a symptomatic or asymptomatic STI (Ct/Ng/syphilis) in the previous 2 years
5. Willing to be enrolled in the cohort for 6 months and attend 3 monthly follow up visits
6. Willing to comply with the mouthwash study schema and willing to ask their casual partners to mouthwash pre- and post-sex
7. Prepared to fill out the online diary once a week
8. Able and willing to provide written informed consent

Potential participants meeting any of the following criteria **will not be enrolled in the study**:

1. Currently using a mouthwash and unwilling to cease use of this mouthwash
2. Enrolment in another interventional trial
3. Tests HIV positive at screening

4.3 Sample size

A total of 320 subjects will be enrolled in the study (160 subjects in each arm). This includes a drop-out rate of 10%. Detailed calculations of the sample size can be found in the statistical section 8.4. We currently have 470 individuals enrolled in our PrEP cohort but this total is expanding rapidly. Almost all are MSM and attend every three months. There is a high compliance with visit schedules partly as they only get a three month supply of medication at each visit.

4.4 Randomization

After all applicable screening assessments have been performed, subjects who have met all inclusion criteria and none of the exclusion criteria will be randomized (1:1) to one of the following groups and will receive a unique randomization number:

1) Listerine ® Coolmint (LCM) mouthwash: daily dose + before and after sex (n= 160)
2) Placebo mouthwash: daily dose + before and after sex (n= 160)

A sub-sample consisting of 60 individuals will be randomly selected to be included for further laboratory examinations related to the *Enhanced Resistome Substudy* (see secondary objectives 9-11). Three different randomization lists will be used – one for the main body of the study (n = 256) and two for the sub-study (2 x n = 32), using block randomization to ensure balance of the two arms among the study sample. The randomization schedules will be prepared by an independent sponsor biostatistician. The overview of the randomization list will not be shared with the investigators until the trial database is locked. The randomization list will be prepared using SAS 9.4 (SAS Institute, Cary NC).

4.5 Withdrawal and termination of the study

Reasons for Withdrawal

In accordance with the Declaration of Helsinki, ICH Good Clinical Practice Guidelines and the Belgian Law on experiments of 2004, a participant has the right to withdraw from the study at any time for

any reason without prejudice to his/her future medical care by the physician at the institution. The Investigator also has the right to withdraw patients from the study if one or more of the following events occur:

- The participant or legally acceptable representative withdraws the consent
- The Investigator judges that further participation would have negative effect on the participant's mental and/or physical health
- The participant tests HIV positive

Handling of Withdrawals

A complete final evaluation should be made at the time of the patient's withdrawal. The Study Status Outcome form in the case report form should be completed with an explanation of why the patient is withdrawing.

Participants withdrawn from study will continue to receive standard of care for their condition. This includes, if needed care of any adverse event or complication, whether related or not to the study procedures. The Principal Investigator (PI) will assure this standard of care is provided and he will discuss specific cases when needed with the Coordinating Investigator.

Participants will be considered lost to follow-up at study closure if he discontinued study visits without informing the study staff and could not be traced. When the investigator has no news of the participant, he/she must make every effort to contact him, to establish the reason for the discontinuation of treatment, and to suggest the participant comes to an end-of-study visit. If all these attempts to contact the participant fail, the investigator can then declare the participant "lost to follow-up". The investigator should document all these attempts in the corresponding medical file.
Participants informing the study staff about their withdrawal from trial will be considered as early withdrawals.

Participants have the right to withdraw their consent at any time and to ask for retrospective withdrawal of all personal data/samples related to the trial.

Withdrawal if participant tests HIV positive

In the event of a study participant testing HIV positive after enrollment but before the 6 month visit they will be withdrawn from the study, their data not used in the analysis and a new participant will be recruited to replace them. If a study participant tests HIV positive for the first time at their 6 month visit which is when the study ends then their data will be used for the study analysis and a new participant will not be recruited.

Termination of Study

The study may be prematurely closed or interrupted by the sponsor in case of futility or adverse health outcomes for the study participants. The decision to interrupt the study will be taken by the study steering committee after consultation of the advisory board.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions, and the regulatory authority of the termination or suspension and the reason(s) for the termination or suspension. The EC's will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5. STUDY PROCEDURES

5.1 Study/visit schedule

Screening and enrollment visit:

- Check inclusion and exclusion criteria (eligibility)
- Review STI history
- Informed Consent Process
- Oral examination + physical examination (as indicated clinically)
- Randomization
- Dispensing of the IMP and training on how to use it (provide instruction leaflet)
- Record antibiotic and non-study mouthwash usage
- Sample collection for the following laboratory procedures:
    - Ng/Ct PCR (first-void urine, anorectal - and pharyngeal regular flocked swabs)
    - Syphilis testing and HIV testing (2.6 mL SST + 7.5 mL SST)
    - Two regular flocked swabs of the pharynx. One swab for microbiome/resistome analysis and if they are selected for the enhanced microbiome/resistome substudy, then one swab for culture of commensal Ng spp.
    - One additional anorectal swabs for microbiome/resistome analysis
- Online questionnaire on sexual behavior

Visit month 3 (range -7 days to +30 days):

- Dispensing of the IMP and training on how to use it (provide instruction leaflet)
- Collect returned IMP bottles
- Record antibiotic and non-study mouthwash usage
- Oral examination + physical examination (as indicated clinically)
- Sample collection for the following laboratory procedures:
    - Ng/Ct PCR (first-void urine, anorectal - and pharyngeal regular flocked swabs)
    - Syphilis testing and HIV testing 2.6 mL SST + 7.5 mL SST)
    - Two regular flocked swabs of the pharynx. One swab for microbiome/resistome analysis and if they are selected for the enhanced microbiome/resistome substudy, then one swab for culture of commensal Ng spp.
    - One additional anorectal swab for microbiome/resistome analysis
- Record serious adverse events
- Online questionnaire on sexual behavior
- Review online diary on adherence to the daily mouthwash and adherence to the pre-post sex mouthwash

Visit month 6 (range -7 days to +30 days):

- Record antibiotic and non-study mouthwash usage
- Collect returned IMP bottles
- Oral examination + physical examination (as indicated clinically)
- Sample collection for the following laboratory procedures:
    - Ng/Ct PCR (first-void urine, anorectal - and pharyngeal regular flocked swabs)
    - Syphilis testing and HIV testing (2.6 mL SST + 7.5 mL SST)

  - One or two regular flocked swabs of the pharynx. One swab for microbiome/resistome analysis and if they are selected for the enhanced microbiome/resistome substudy, then one swab for culture of commensal Ng spp.
  - One additional anorectal swab for microbiome/resistome analysis
- Record serious adverse events
- Online questionnaire on sexual behavior
- Review online diary on adherence to the daily mouthwash and adherence to the pre-post sex mouthwash

Unscheduled visits:

- Visits for treatment of detected STIs
  If any STI is diagnosed via screening (including Ct, Ng, syphilis, HIV or hepatitis C) at any stage during the study, the current practice of contacting the PrEP recipient (typically by telephone) as soon as the test result is known (typically within 7 days) will be followed. The person will be asked to return to the clinic at their earliest convenience for standard management, including contact tracing. At this stage if the person had a positive molecular test for Ng they will have swabs taken for Ng culture from each site that was positive for Ng. These STIs will be treated according to our current guidelines [96-98].

  Persons with an HIV diagnosis will be offered optimal antiretroviral therapy and further best practice management according to our existent PrEP protocol. They will exit this study after the diagnosis of HIV is made. In case participants are tested HIV positive during their final visit then their data will be used entirely. In any other case of HIV infection, the participant will be replaced by a new enrollee.

- As is currently the case for all PrEP recipients, participants of this study will be able to attend the ITM clinic at any point in between the scheduled visits for any health related concerns. Participants will be encouraged to attend the clinic for any symptoms compatible with an STI including pain or other symptoms in the oral cavity and pharynx. Any STIs (Ng, Ct or syphilis) diagnosed at these non-scheduled visits between 0 and 6 months will be included in the primary outcome.

- Post-trial visits:
  Any patient who has any symptoms which they consider to be related in any way to the use of the IMP will be encouraged to return for a detailed assessment at a post-trial visit. These will be recorded as unscheduled (post-trial) visits. These may occur until 3 months after the patients last study visit.

A schematic overview of all study assessments can be found below in table 1.

Table 1: Study assessments according to study visit

Procedures	***Screening / Enrollment (V1)***	***Visit Month 3***	***Visit Month 6***	***Unscheduled visits (USV)******
STI history	x			
Eligibility	x			
Informed Consent	x			
Randomization	x			
IMP dispensing	x	x		x
Oral examination *(+physical examination if indicated)*	x	x	x	x
Antibiotic and non-IMP mouthwash usage	x	x	x	x
Serious Adverse Event collection		x	x	x
Pharyngeal swab 1 Molecular Ng/Ct Testing	x	x	x	x
Pharyngeal swab 2 Microbiome/resistome analysis	x	x	x	
Pharyngeal swab 3* Culture commensal *N spp*	x	x	x	
Anorectal swab 1 Molecular Ng/Ct Testing	x	x	x	x
Anorectal swab 2 Microbiome/resistome analysis	x	x	x	
Urine Molecular Ng/Ct Testing	x	x	x	x
Ng culture*	x*	x*	x*	x*
Blood Serum RPR/TPA	x	x	x	x
HIV	x	x	x	x
CASI questionnaire on sexual behaviour & mouthwash use	x	x	x	x
Review online diary* ***- Adherence to daily mouthwash*** ***- Adherence to pre-post sex mouthwash***		x	x	x

***** *ONLY if any site (urine, pharynx or rectal) is Ng PCR positive*

***The online diary will be completed weekly*

**** As clinically indicated*

*****Only performed on those selected for the enhanced resistome/microbiome substudy (60 subjects)*

5.2 Obtaining informed consent

The Informed Consent Form (ICF) documents will be designed in accordance with the requirements of the Helsinki Declaration (2013), the E6 ICH GCP Guidelines (2016) and the Belgian Law on Experiment on the Human Person (2004). The ICFs will be developed in Dutch, French and English. The translation(s) will be reviewed and translation validation forms will be completed and signed by both the translator(s) and reviewer(s). The IC procedure will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, confidentiality issues, etc.
All informed consent procedures will be conducted by qualified staff members identified by the principal investigator and done in the language chosen by the participant. Participants will be informed that participation in the study is completely voluntary and that they may withdraw from the study at any time without any negative consequences. Participant Information Sheets and consent forms will be provided to the study participants for review. The participants will be given enough time to consider whether or not to participate in the study. Upon agreement on participation, the consent form will be signed in two copies, namely by the participant and by the investigator administering the consent. The participant will receive one copy of the ICF, while the other copy will remain in the Investigator file. If a participant is unable to read or write, a signature from a witness to the informed consent discussion will be obtained.
No participant may be enrolled in the study until the study investigator or designee has obtained his informed consent. The qualitative research sub-study will follow a separate Informed Consent and Participant Information Procedure (see section 9).

The ITM SOP for 'Obtaining the Informed Consent of clinical trials' subjects will be followed.

Casual sex contacts: Participants will be asked to perform the mouthwash step with casual sex contacts. Casual sex contacts are defined as all non-main sex partners. During all study visits, participants will be asked to identify with initials who their main partners are. All partners who are not defined as main partners will be defined as casual partners for the purpose of the study.

Participants will be asked to inform casual sex partners about the study rationale and methods. They should then ask the partner if they would be willing to mouthwash pre and post sex. If they decline then the participant will be requested not to pressurize the partner to use the mouthwash pre- and post-sex. It will be emphasized that if the partner declines to use the mouthwash this should not deter them from having sex with this individual. Participants will be provided with flyers containing information pertaining to the study which they can give to their partners if they find this useful. The flyer will contain contact details of the study team. This will enable the partners of the participants to acquire more information pertaining to the study as needed. This flyer will be submitted and approved by the Ethical Committees before use in the study.
Main partners: It will be explained that although the participant may choose to conduct the pre and post sex mouthwash with their main partner(s), that this is not expected in the study.

5.3 Specific procedures and activities

The following study specific activities will be conducted at each study visit:

***Antibiotic and non-IMP mouthwash usage*:**

- Additional study questions pertaining to mouthwash use in last 3 months, antibiotic use in last 3 months, STIs diagnosed in last 3 months and any oral health problems. These questions will be asked via a Computer Assisted Structured Interview.

Oral examination:

- Caries: an SOP will be available to guide the investigators for a coherent assessment
- Tooth staining: an SOP will be available to guide the investigators for a coherent assessment
- General changes of mouth mucosa: as reported by the participants

Physical examination:

- Physical examinations as indicated clinically.

Collection of swabs:

- The study physician will take oropharyngeal and anorectal samples according to clinical SOPs. If the participant elects they may take the anorectal sample by themselves.

Collection of first-void urine sample (collected by the participant).
Collection of blood sample (by trained personnel). 2.6 mL SST + 7.5 mL SST.

5.4 Laboratory procedures

All laboratory procedures except for microbiome and resistome testing will be performed at the ITM. At every visit, blood, urine and anorectal and pharyngeal swabs will be taken from every participant. In addition, at all visits extra pharyngeal and anorectal swabs will be taken for resistome/microbiome profiling.
Please refer to section 5.1 Study procedures when samples are taken and below-mentioned assays are performed.

5.4.1. Laboratory testing performed at ITM

Laboratory testing at the ITM is always performed according to their validated SOPs.

Testing at the HIV/STI Reference Laboratory

- HIV testing will be performed according to the ITM algorithm as in routine practice
- CT/NG molecular testing will be performed at the STI Reference Laboratory on urine, anorectal and pharyngeal samples. In case an anorectal sample is found to be positive for CT, an additional test will be performed to differentiate L versus non-L strains.
- Culture of commensal pharyngeal Neisseria species

Testing performed at the CLKB

- Syphilis testing will be performed according to the ITM algorithm as in routine practice
- Culture of *Neisseria gonorrhoeae* in case Ng was detected using molecular techniques

5.4.2. Laboratory testing at external laboratories

Resistome and microbiome profiling will be performed at Laboratory of Medical Microbiology, University of Antwerp. All swabs taken for that purpose will be stored at the STI Reference Laboratory at -80°C. At the end of the study, the samples of the 60 individuals designated to participate in the enhanced resistome/microbiome substudy will have their 0, 3 and 6 month pharyngeal microbiomes and resistomes profiled according to the procedures outlined in Appendix 1.

6. STUDY INVESTIGATIONAL PRODUCT

6.1 Purchasing, preparation and administration

The study will use two IMPs:

1) Listerine ® Coolmint (LCM) mouthwash – Johnson & Johnson

   Active ingredients: Eucalyptol (0.092%); Menthol (0.042%); Methyl salicylate (0.060%); Thymol (0.064%)

2) Placebo mouthwash

   Ingredients (per 200mL):
   - Sorbitol 30g,
   - Sodium saccharin 0.1g,
   - Solution Vitris Nf5 15 drops
   - Aq Conservans Nf4 Ad 200g

LCM has EC-marketing approval and is commercially available without prescription in pharmacies and supermarkets. SmPC documentation is available for this product.

LCM will be purchased by the sponsor and shipped to the designated pharmacy where it will be re-packaged in blinded bottles and re-labelled for the study. Once packaged and labelled, the bottles will be transported to the ITM.

The placebo will be manufactured at the designated pharmacy, where it will be re-packaged in blinded bottles and labelled for the study. Once packaged and labelled, the bottles will be transported to the ITM.

At every study visit, study subjects will be provided with an amount of IMP that will be sufficient for the next 3 months. Frequency of administration will be:

- Daily dose of 20mL (gargle for 60 seconds) once a day
- Together with casual sexual partner before sex and after sex (up to 6 hours thereafter) with 20mL of the product (gargle for 60 seconds).
- Participants should not use LCM more than 5 times per day in accordance with the package insert

Additionally, sexual partners of study participants will be asked to use the mouthwash pre and post sex. The different scenarios are described in the below table.

Scenario	**Administration procedure**
Study participant has sex with his ***main*** partner and they have decided to mouthwash pre and post sex *	Both individuals use the mouthwash pre and post sex **
Study participant has sex with his ***main*** partner and they have decided ***NOT*** to mouthwash pre and post sex *	Neither the participant nor their partner uses the mouthwash pre and post sex **
Study participant has sex with his ***main*** partner and they have decided that the study participant but not the partner will mouthwash pre and post sex *	The participant but not their partner uses the mouthwash pre and post sex **
Study participant has sex with a ***casual*** contact NOT enrolled in the study	Both study participant and casual contact use the mouthwash **
Study participant has sex with a ***casual*** contact ALSO enrolled in the study	Both study participant and casual contact use BOTH mouthwashes **
Study participant has sex with a ***casual*** contact and the contact decided ***NOT*** to use the mouthwash	Only the study participant uses the mouthwash pre and post sex **

* *If a main partner is also enrolled in the study then the same principle applies. They will only mouthwash pre and post sex with one another if they have decided that this is what they want to do.*
** In all scenarios, the study participant continues with the daily mouthwash step.

An SOP will be available describing the IMP administration procedures. Additionally, instruction leaflets will be available for all subjects.

6.2 Participant adherence monitoring

It will not be possible to verify complete adherence to the IMP since the administration will not be directly observed. However self-reported compliance will be queried using a weekly online diary that study subjects will be asked to complete. Subjects will be trained and reminded about the importance of good adherence at every study visit.
Study participants will be asked to return all study bottles (empty, full and partially full) at their next study visit. The remaining volume will be reported in the eCRF (and compared with the reported usage in the diary) as part of compliance monitoring.

6.3 Prior and concomitant therapy

Participants will be asked not to use any other mouthwash than the study product during the study. They will be asked at all study visits if they have used any other mouthwashes and antimicrobials in the preceding 3 months. This information will be noted in the participant file and reported in the eCRF. They will be able to take other medications and drugs. Information as to these additional medications will not be collected.

6.4 Packaging

After purchasing the products in the commercial packaging, the products will be shipped to the designated pharmacy where they will be re-packaged in blinded bottles. All blinded IMP bottles will then be labeled with trial specific labels each containing a unique IMP randomization number. Only the pharmacy and the independent statistician that prepared the randomization list will have access to the allocation list with all randomization numbers.

An emergency unblinding procedure will be available to the Investigator and to designated persons at the Sponsor. Breaking of the blind for individual patients in emergency situations is an Investigator responsibility. As far as the emergency permits, the need to break the blind will be communicated to the Sponsor.
The unblinding in emergency situations is only permitted in case of an important adverse event, when the knowledge of the IMP in question is required for therapeutic decisions for the management of the patient. As far as the emergency permits, the need to break the blind will be agreed by the Investigator and the Sponsor. The Investigator who unblinds a treatment must record the reason and date for unblinding before the treatment code can be broken. The Investigator must record the event of unblinding in the patient's medical record, including the reason for unblinding, but not the treatment allocation if this can be avoided.
In case of accidental unblinding, the same documentation as for emergency unblinding must be obtained.
If the Sponsor needs to unblind a treatment, the reason and the date of opening should be recorded with signature, following corporate standard operational procedures for unplanned unblinding of clinical trial patients. It should be recorded in the subject's source documents that the code is broken, why, when and by whom.
If it is necessary to unblind an individual patient's treatment for the purposes of expedited reporting to the IECs, only those individuals within the Sponsor whose responsibility it is to report this information will know the identity of the IMP. Every attempt will be made to ensure that all other trial and site staff will remain blinded throughout the course of the trial.
Information on whether the blind has been broken for any patients must be collected before the database is declared clean and is released to the statistician.

6.5 Reception, storage, dispensing and return

After re-packaging and labeling at the pharmacy, the products will be transported to the ITM where they will be stored in the pharmacy room at ambient temperature. This room is locked and accessible only for study staff. The room temperature is monitored using a min-max thermometer that is reviewed daily (during the week).

Receipt of the products will be documented on an inventory log kept by the study coordinator. Distribution of IMP bottles to the subjects will be documented on a subject accountability log that will also be managed by the study coordinator. This will include documentation as to what volume of IMP is left over at the end of each 3 month period (Study participants will be asked to return all study bottles (empty, full and partially full) at their next study visit).

In case of damage or expiry of any product, the product will be placed in quarantine by the study staff and this will be documented on the inventory log. Pharmacy staff will be contacted for review and replacement of the quarantined products.

7. SAFETY ASSESSMENT

(Serious) Adverse Events will be monitored and recorded starting from the enrollment visit (start of the study mouthwash use) and up to the last visit (month 6 visit, last day of study mouthwash use).

7.1 Adverse events

Since the IMP is already marketed and widely used [92], a predefined list of adverse events (as listed below) will be reviewed at every study visit and reported in the source documents. These events will also be recorded in the eCRF:

1. Teeth staining
2. Any new oral symptoms (Open question)

Serious Adverse Events

A Serious Adverse Event (SAE) is any adverse event/experience occurring at any study drug dose that results in any of the following outcomes:

- Death;
- Life threatening (participant at immediate risk of death);
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant disability or incapacity;
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All SAEs must be:

- recorded on the appropriate SAE report form
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician.
- Follow-up until resolution (by sending updates on the SAE form)

SAEs will be collected from the start of the intervention until month 6 (end of intervention).

Severity, relationship of event to study drug and outcome

All SAE's will be assessed by the clinician using a predefined grading system:

1. **Mild:** events require minimal or no treatment and do not interfere with the participant's daily activities.
2. **Moderate:** events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
3. **Severe:** events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
4. **Life-threatening:** Participant at risk for death at the time of the event

Changes in the severity of an SAE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Assessment of causality

The investigator is obliged to assess the relationship between investigational product and the occurrence of each SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the IMP will be considered and investigated. The investigator will also consult the drug information and the DSMB as needed in the determination of his/her assessment.

The relationship of an adverse event to study drug is to be assessed according to the following definitions and can only be done by the study physician:

1. **Definitely unrelated**: Reserved for those events which cannot be even remotely related to study participation (e.g. injury caused by a third party).
2. **Unlikely**: There is no reasonable temporal association between the study drug and the AE and the event could have been produced by the participant's clinical state or other modes of therapy administered to the participant.
3. **Possible**: The suspected AE may or may not follow a reasonable temporal sequence from study drug administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the participant's clinical state or by other modes of therapy concomitantly administered to the participant.
4. **Likely**: The suspected adverse event follows a reasonable temporal sequence from study drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the participant's clinical state.
5. **Definitely related**: Reserved for those events which have no uncertainty in their relationship to test drug administration: this means that a re-challenge was positive.

The outcome of each SAE must be assessed at the same visit and according to the following classification:

Recovered: The participant recovered from the event with no residual problems.

Not yet recovered: This outcome can only be used for Serious Adverse Events. The event no longer meets a 'Serious' criterion, but medical event is not yet completely cured. The remaining event should be listed as a separate adverse event in the adverse event table, and with its own outcome.

Permanent damage: The event has resulted in permanent impairment.

Ongoing: the participant is continued to be followed for the event.

Death: The participant died. This term should only be used for the event which resulted in death. Any other events which were present at the time of death, but were not the cause of death, should be listed as 'Ongoing'.

Unknown: The participant cannot be traced and no final outcome for the event could be determined.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always

makes an assessment of causality for every event prior to transmission of the report to the Sponsor. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE-report form accordingly.

All SAE's whether or not deemed drug related or expected must be reported immediately or within 24 hours (one working day) using the SAE Notification Form by email to:

Clinical Trials Unit
Institute of Tropical Medicine
Nationalestraat 155
B-2000 Antwerp – Belgium
Email: pharmacovigilance@itg.be

Line listings of all reported SAE's will be sent to the IRB of the ITM and the EC of UZA on a yearly basis. They will also be sent yearly to all recruitment sites in order to submit these to their respective Authorities if necessary.

It is the Investigator's responsibility to adequately and timely report Adverse Events to the local EC's in compliance with applicable local regulations.

A study safety checklist SOP will be prepared describing all responsibilities and actions that are required in case of an SAE.

7.2 Independent Safety Monitor

A Data Safety and Monitoring Board is not mandatory for this type of project, where the IMP is an already marketed and widely used product (non-medicine). However, to ensure an extra layer of independent supervision on safety issues, the Sponsor will appoint a data safety monitor, who is fully independent from the study team. He/she will receive and review all SAE reports on an ongoing basis. A Charter will be set up to describe the Safety Monitor role. The independent data safety monitor will be experienced in STI medicine and clinical research. In case of major safety concerns, this independent safety data monitor may advise the sponsor to halt recruitment of the trial and/or request to organize a formal meeting with the TMG members with a complete overview of all available safety data.

8. STATISTICAL METHODS

The statistical analysis will be described in the statistical analysis plan (SAP), written by the biostatistician, which is binding and will be finalized before database lock or before any other analysis takes place.

8.1 Study hypotheses and hypothesis

The primary hypothesis of this study is that LCM mouthwash used daily and pre/post sex is able to reduce the incidence rate of all of the three infections of interest (Ng, Ct, syphilis) in MSM over a 3 month period.

8.2 Variables of interest

Primary: An indicator variable counting the cumulative number of infections (Ng, Ct, syphilis) diagnosed in each study subject. The diagnosis of Ng and Ct will be made via molecular testing and syphilis via RPR and TPA tests according to currently used European case definitions [96].

Secondary:

- A binary variable indicating a pharyngeal Ng infection for every patient
- A binary variable indicating a Ct infection for every patient
- A binary variable indicating a syphilis infection for every patient
- Three ordinal variable indicating the percentage of compliance with the recommended dosage for every occasion (daily, pre- and post-sex)
- The count of different antibiotic courses used over each treatment period by each participant
- The count of patients experiencing an adverse event

The level of adherence will also be transformed into a binary/categorical variable with pre-specified cut-offs in case of sparse representation in the original categories.

8.3 Statistical methods

8.3.1 Analysis populations

The primary analysis and secondary efficacy analyses will be performed using an intention-to treat approach. In addition, an all patients-treated analysis will be performed where all participants randomized but never starting the study products will be analyzed.

8.3.2 Baseline characteristics

The number of participants screened and enrolled or excluded will be summarized according to reason for exclusion. Of the enrollees, the number of patients discontinued or lost to
follow-up will be recorded by reason and time of discontinuation. These figures will be summarized in a CONSORT flow diagram.

Patients in each treatment group will be described according to baseline characteristics. The description will be in terms of medians and interquartile ranges for continuous variables and using counts and percentages for categorical variables. Standard statistical tests of significance of imbalance in baseline characteristics will be performed.

8.3.3 Primary analysis

A mixed effects Poisson regression model will be fitted with sum of Ng, Ct and syphilis infections occurring in one person as the outcome variable, treatment (placebo or LCM), time on LCM/placebo treatment (number of days above or below the reference 90 days [3 months] on LCM or placebo) and visit as independent variables and a random intercept. The primary objective will be addressed through

the p-value of the treatment variable in the model.

In a pre-specified analysis we will repeat this analysis but controlling for adherence to LCM mouthwash and different time periods on the LCM vs. placebo.

8.3.4 Secondary and tertiary analysis

Secondary analyses

The secondary objectives regarding the individual infections will be analyzed in a similar way as the primary analysis, using mixed effects logistic regression models instead of Poisson. In the analysis controlling for adherence levels the model of the primary analysis will be used, adding an extra covariate for adherence. The secondary objective regarding antibiotic use will be analyzed either using mixed-effects Poisson model with treatment and time as covariates or using Chi-square test to compare the proportions in the two groups at different time points. All lab-related (microbiome/resistome) objectives will be analyzed by comparing study arms with the Wilcoxon rank sum test for continuous data and the Chi squared or Fischer's exact test for categorical data. The patient count with adverse events in the two groups will be compared using Fisher's exact test.

STERGM modeling: Modeling the probability of emergence of AMR in Ng
Capturing the emergence of AMR in NG in a PReGo-type study using LCM vs. placebo would require a combination of large sample sizes/long follow up/multiple reinfections. As a less costly alternative, we plan to model the probability of the emergence of AMR in scenarios with varying levels of LCM mouthwash usage. We are currently adapting our Separable Temporal Exponential Random Graph (STERGM) model of Ng transmission in MSM in Belgium[33] to include alterations in each person's resistome following antibiotic treatment with specific decay curves based on empiric data. In the model, incident Ng is able to acquire certain prevalent resistance mutations (via transformation and spontaneous mutations) based on parameters such as antibiotic usage/alterations to the resistome. This model will allow us to estimate the probability of the emergence of resistance to ceftriaxone/azithromycin in two scenarios driven by the results of our LCM mouthwash trial (Secondary outcome 11). If the LCM arm has a lower incidence of STIs, lower antibiotic exposure and lower transit of Ng through the pharynx (a key site for AMR acquisition) then we will be able to model to what extent these differences would reduce the emergence of AMR in MSM in Belgium if various proportions of MSM utilized the LCM intervention.

8.3.5 Subgroup analyses
A qualitative research substudy will be performed as detailed in '9. Qualitative research methods'.

8.3.6 Multiplicity and Missing Data
No adjustment for multiplicity are needed. This is a study where every participant is measure 3 times. The differences among study subjects and within them in the different time points are corrected in the model of the primary endpoint. No interim analyses are planned. We estimate that 5% of visits will be missed. Since we will not be able to assess for STIs at these visits, these visits will be dropped from the analysis.

8.3.7 Interim analysis

The questions on acceptance and adherence from the questionnaire at month 3 will be analyzed every two months with the purpose of selecting 20 participants for a semi-structured interview (see section 9). Selection of the participants will be based on 4 criteria relating to acceptability and adherence (section 9.2). If needed, the questions of acceptance and adherence from the questionnaire at month 6 may be also be analyzed in the same manner, for the same purpose.

8.4 Sample size and power

We assume that the PReGo LCM mouthwash protocol will result in a 50% reduction in incidence of Ng and *T.pallidum* and a 30% reduction in Ct incidence [32]. These figures are based on a previous study that found a five-fold reduction in pharyngeal Ng following mouthwashing with LCM [32] and *in vitro* studies that show that Listerine based essential oils are similarly bactericidal against Ct and *Treponema spp*. as they are against Ng [82-84,89]. Based on incidence rates of Ng (10.6%), Ct (11.1%) and *T.pallidum* (1.5%) in our ITM Be-PrEP-ared study (N=200) [35] we expect an incidence rate of 13.8 cases and 23.2 cases per 100 study visits in the LCM and placebo arms respectively for any infection with Ng, Ct or *T.pallidum*. Correcting for a drop-out rate of 10%, we will need 160 men in each arm to detect this effect size with 90% power using a two-sided significance level of α=0.05. The sample size was calculated through simulation using the statistical software R (citation: R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

9. QUALITATIVE RESEARCH METHODS

9.1 Qualitative research approach

Mixed method design

A qualitative study will be embedded into the trial to address secondary objectives 6 and 7, i.e. exploring the experienced and perceived barriers and facilitators for optimal mouthwash adherence among MSM taking PrEP (=objective 7) and examining whether and how mouthwash is acceptable as a method for preventing STIs among MSM taking PrEP (=objective 6).

The qualitative will complement the quantitative research methods (i.e. questionnaire data) that are used throughout the trial. The qualitative study will provide further in-depth insights into participants' perspectives on their experiences of using mouth-wash. The qualitative research will be used to corroborate and if needed, further explain, the quantitative results with qualitative findings, giving way to a [QUAN+qual] concurrent nested mixed method design' [99]. Such triangulation of insights and research methods will further enhance the validity of the findings.

Data collection method: Semi-structured interviews

Face-to-face interviews are the most appropriate data collection methods, since we are interested in personal experiences, which may be related to sensitive issues such as sexual risk and protection behaviour [100]. We will use semi-structured interviews, as they allow us to collect data in a structured, comparable manner, while ensuring the flexibility to identify new ideas or to explore given aspects of the topic more in-depth.

Qualitative research team

The qualitative research will be undertaken by one social science researcher who is experienced in qualitative data collection and with knowledge of the subject. A small interdisciplinary research team will be set up for this sub-study, including one psychologist, sociologist, anthropologist and the coordinating investigator. The research team will co-analyse the data and insights, thus ensuring multiple perspectives in the process and further enhancing the validity of the findings.

Inductive approach

Data collection and analysis will be guided by a constant comparative analysis approach: an iterative process of comparing data to confirm or contrast evidence until no new insights are found [101]. The preliminary quantitative findings of the study will be used as a basis to develop the interview guide. Data collection and concurrent analysis will be focused on developing insights and theory out of the data collected.

9.2 Participant recruitment

Participants for these semi-structured interviews will be purposefully selected from the PreGO trial. We estimate that about 20 interviews (and hence 20 participants) will suffice to reach data saturation, i.e. no new insights will emerge from the interviews, but more can be collected if the qualitative research finds that data would not be saturated after n=20 interviews.

We will purposefully select participants based on their answers in the questionnaire(s). We consider that identifying and interviewing such 'rich cases' will yield better insights into the possible range of barriers and facilitators regarding the use of this STI prevention method (endpoint 7). It will also generate knowledge on as to why participants may or may not find the prevention method acceptable. We will purposefully select four types of participants:

a) Those indicating the study drug to be 'highly acceptable' as an STI prevention method
b) Those indicating the study drug to be 'highly unacceptable' as an STI prevention method
c) Those who indicated to have had 'very good adherence'
d) Those who indicated to have had 'very bad adherence'

Participants will be considered as a 'rich case' when they are in the upper or lower decile of the 'acceptability' and 'adherence' variables.

9.3 Data collection

Interview scheduling

Participants will be asked by the study coordinator whether they may be contacted by the social science research of this sub-study to schedule an interview. If potential participants consent, the social science researcher will schedule an interview on a time and place that is most convenient for the participant. Most likely, the interviews will be undertaken before or after visiting the clinic for follow-up visits (e.g. at month 6), at the study site to reduce organizational threshold for participation. Interviews may be scheduled outside of the study visits or study site, only if the participant considers this not to be feasible otherwise.

Informed consent

Participants will need to provide additional consent for conducting the semi-structured interview. The following topics will be covered before starting the interview: the purpose of the interview, approval for the use of the audio recorder will be asked, how the interview data will be used and stored, that participation is voluntary, the participant may quit or not respond to a question at any time and if questions are too sensitive or unclear the participant is encouraged to say so.

After the information has been provided, the participant will be asked whether he agrees on participating. The oral consent will be recorded on the audio tape.

Interview guide

An interview guide will be developed for the purpose of this study. The interview guide will be developed on the basis of the findings of the quantitative research by the interdisciplinary qualitative research team. Input from the community advisory board will be obtained. The interview guide may be adapted in between interviews (after preliminary analysis), as it is part of an iterative research method and crucial for an inductive approach. Adaptations will only be done in collaboration with the qualitative research team and without compromising consistency.

Data collection phases

Data collection will follow three subsequent phases. For each of the four 'rich case types' two cases will be interviewed (phase 1). After a first analysis (see analysis) a second round of two interviews will be undertaken per case type to look for novel insights or to confirm those already found (phase 2). In a last round, one case for each 'rich case type' will again be interviewed (phase 3), totalling to a minimum of 20 interviews. If data should not be saturated, a fourth, fifth, … phase may be undertaken, depending on the data.

Recording and transcripts

All interviews will be audio-recorded and transcribed verbatim. While transcribing the transcripts it will be ensured that no information is present in the transcription that can be used to identify the participant. A pseudonym will be used to this extent. A representative proportion of these transcripts will be checked manually at random for accuracy, by listening to selected sections of the audio-tapes and subsequently comparing them with the transcripts. If needed, the transcripts will be improved. This quality check will be done prior to the data analysis (coding of data).

9.4 Data analysis

All data will be analysed using a computer-assisted software program for qualitative data analysis (e.g. NVivo or comparable programs). An inductive coding process following a grounded theory approach will be used [101]. Three parallel processes of data analysis are used in between and after collecting the data: open, axial and selective coding. It implies that the transcripts will be iteratively scrutinized for emerging themes to develop a coding scheme that focuses on reaching the endpoints as described earlier (endpoints 6 &7).

'Open coding' focuses on categorizing and describing the data, by developing key concepts and categories. A data-driven coding framework will be developed by the responsible social science researcher, in collaboration with at least one team member of the qualitative research team, to ensure that no insights are missed. The coding framework will then be used to adapt the interview guide, where necessary to explore new or contrasting evidence, without compromising consistency. Such open coding will be undertaken after every phase of data collection. In this study for example, open

coding will mostly pertain to describing the different barriers and facilitators that have come up during the interviews.

A second analysis process is 'axial coding', which focuses on finding relations and patterns. While open coding focuses on finding differences and similarities, axial coding provides more insights about these differences and similarities. In this study for example, axial coding may focus on finding patterns in participants' perceptions regarding their experienced barriers and facilitators with regard to the use of the study drug.

A third and final analysis process comprises 'selective coding', which focuses on developing theory. In this step, the patterns and relations found will be used to develop insights on a particular idea or finding. For example, in this research, insights on what participants perceive to be a feasible STI prevention method, as well as how this pertains to their sexual risk behaviour, will be used to develop a theoretical explanation on the acceptability of the study drug.

9.5 Qualitative Data management and storage

During data collection, multiple audio files may be present (i.e. on the recorder, as well as on the personal encrypted, password-protected server of the social science researcher), to ensure that no data will be lost. All such audio files will not be accessible without password, or remain locked when not in use (i.e. the recorder). After all interviews are transcribed, the audio files will be archived, locked and stored on a personal server of the social science researcher as a back-up, along with the audio versions of the informed consent. All other audio files will be deleted after this step.

The transcripts and other interview data (notes and coding frameworks) will be available on an interdepartmental server account of the trial, which is only accessible by the researchers who are involved in this study.

10. MONITORING AND QUALITY ASSURANCE

This study will be monitored in accordance with regulations applicable to clinical trials, including ICH-GCP and WHO-GCLP, and sponsor-specific SOPs. The PI and involved site research staff will allocate adequate time and resources for such monitoring activities. The investigator will also ensure that the monitor or QA reviewer is given access to all the above noted study-related documents and study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) and has adequate space and resources to conduct monitoring and source data verification.

A monitoring plan will be written to describe monitoring responsibilities and activities in detail (including percentage of SDV, timing and frequency of site visits, follow-up of findings and protocol deviations).

The sponsor will inform the Investigators concerned immediately upon notification of a pending study centers inspection by any regulatory authority or funder. Likewise, the investigator will inform the sponsor of any pending inspection.

Laboratory quality control and quality assurance:
ITM will ensure that all laboratory activities including specimen transport, processing, testing, result reporting and storage will be conducted in accordance with the clinical trial quality requirements. The

laboratory will perform testing according to the SOPs which are documented in the laboratory analytical plan and will be conducted in compliance with Good Clinical Laboratory Practice Standards (GCLP), and EN-ISO 15189. Reports of laboratory test results will be forwarded to the study physician as soon as the result is available.

11. DATA MANAGEMENT

Due to the integration of quantitative and qualitative methods in this study, different type of databases will be set up:

- A clinical database will be programmed and validated prior to study start
- Online diary on adherence to daily and pre-post sex mouthwash
- CASI questionnaire on sexual behavior
- Questionnaire on the acceptability of the mouthwash

The participants will be identified by a study specific participant number and/or code in a database (pseudonymisation). The name and any other identifying detail will be retained at the recruitment site only and will NOT be included in any study data electronic file.

The Investigator will retain all source documents for each participant in the study, laboratory data, questionnaires, and the results of any other tests or assessments as well as all other essential documents.

11.1 Data management clinical data

Types of data collected

Individual, participant-level clinical trial data will be collected by the clinical site team and held in patient files at the site. Collected data will include variables from Baseline visit (de-identified trial participant code, eligibility screening, informed consent confirmation variables, medical history, concomitant medication, randomization, vital signs, lab test results, and adverse events data), Follow-Up visit data (lab test results, concomitant medication, (serious) adverse events data) and Outcome data (final outcome).

Data will be entered at the sites via study computers equipped with electronical case report forms (eCRFs) developed with MACRO, a GCP- and regulatory compliant clinical trials management software. Testing and validation of the eCRF design, including data quality checks, will be documented.

Data provisioning

- Responsible person

  Data Management will be performed by the trial staff at the site, in collaboration with a data manager from the Clinical Trials Unit (CTU) at the ITM.

- Storage & preservation of data

All the relevant study data will be retained for a minimum of twenty years and according to applicable regulations. The trial computers and eCRFs will only be accessible via a Login with personal username and password. A list of authorized users of the eCRFs will be kept at the CTU and updated regularly.
The trial database will be stored on a secured server at the ITM, which is physically only accessible by badge by authorized IT collaborators. Access to the study database is limited to CTU data managers. The ITM has procedures in place to ensure daily backup of the server and study computers and for long-term, secure curation and preservation of data.

- Confidentiality & security

  Information of trial participants will be handled confidentially. A trial participant code will be assigned to each trial participant. Any information that could lead to the identification of the participant will not be included on the eCRFs, nor on any other paper documents or electronic files used for data management. The name and contact data for each participant will be kept separately and limited to authorized staff at the sites.

- Other issues

  Data Management will be done in compliance with Good Clinical Practice (GCP) guidelines and FDA 21 CFR part 11 regulations and will include a robust security system including SOPs, encryption, restricted access, daily back-up and traceability.

  A study specific data management plan and data validation plan will be developed. The Data Management Plan will describe the lifecycle for the data to be collected (clinical data and questionnaire data), processed and/or generated and includes information on how data will be collected, processed, shared, curated, preserved and includes the methodologies and standards applied. The Data Validation plan will describe the process of making data complete, accurate and consistent i.e. ensuring data quality.

  Medical event terms will be coded and standardized using the Medical Dictionary for Regulatory Activities (MedDRA).

  Metadata (e.g. variable name, variable description, label, data type (date, string, number), etc.) will be kept in a data dictionary.

  After study completion and publication of results, anonymized or pseudonymised individual participant data may be shared by means of a managed access procedure. To this end, the ITM Data Sharing Policy will be adhered to.
  All research data management will be performed in compliance with the European General Data Protection Regulation 2016/679 (GDPR).

11.2 Data management qualitative data

All qualitative research data will be gathered in password-protected server-located folders for the different qualitative research sub-studies. The social science researcher will keep a log file to

document the data collection process (including interview notes, transcripts, memos, etc.). To ensure the participants' confidentiality, only the researchers will be able to access these documents.

11.3 Data management of questionnaire and diary

Personal data collected in the questionnaire and the diary will be pseudonymized as much as possible. Study participants will use a secure login and password to enter data. Access to data in the databases will be restricted to the designated study staff. Specific security and data protection measures will be detailed in the Data Management Plan and will be validated prior to data entry.

12. ETHICAL ISSUES

The study design that includes asking casual partners to perform the mouthwash pre and post sex was developed to balance a number of considerations. As outlined above if the participants only mouthwash daily this would not give them the maximum protective benefit from the mouthwash. This is because it would not protect them from acquiring STIs from their sex partner's pharynx - particularly those acquired in the participant's rectum and urethra. If they get their partner to mouthwash pre and post sex this will enhance the protective effect of the intervention. It will also protect their partner in the same way. The partner will not however sign an informed consent form. Instead the participant will be asked to inform the partner about the rationale for the study and the risks and benefits of participation. A leaflet will be produced to give them an outline of how they could provide this information. This leaflet could be given to partners as desired and include contact information of the study coordinators if the partner had further questions. The partner would be informed that their use of the mouthwash was voluntary and they could chose to use it pre and post sex, either pre or post sex or not at all. These guidelines were worked out following discussions with members of the community advisory panel including PrEP users. We took into account the fact that a number of MSM are currently using a range of over the counter mouthwash preparations to prevent them acquiring STIs. Participants who are before study participation using a mouthwash that is effective against STIs may be placed at an increased risk for STIs during the period of the study when they use the placebo. This risk will be explained in the ICF

12.1 Ethical and regulatory review

This clinical trial will be submitted for formal review and approval to the Institutional Review Board of the ITM, the EC of the University Hospital of Antwerp. No study-specific interventions will take place before written approval by the Ethics Committee(s) has been obtained and the local regulatory requirements have been complied with, and the signature of the clinical study protocol of each contractual party involved have been obtained.

The study will be carried out according to the principles stated in the Declaration of Helsinki, all applicable regulations and according to the most recent GCP and GCLP guidelines.

12.2 Protocol amendments

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the appropriate steps before being implemented. Any substantial change must be approved by all the bodies and EC's that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns (in which case the immediate implementation can be necessary for the sake of participant's protection. In case modifications to the protocol or amendment are

requested by any local EC during the review process, these must be discussed and agreed upon with the Sponsor prior to any resubmission incorporating those changes.

12.3 Informed consent

No participant may be enrolled into the study until the Investigator or designee has obtained the written informed consent form. For detailed ICF procedures, see section 5.2, and 9.3, respectively.

12.4 Confidentiality

All the participant data will be pseudonymised in all collection tools, the CRF and database by means of a unique subject assigned participant study number. The documents which can identify the participants, e.g. the ICF's, laboratory print-outs and medical record, will only be accessible to the relevant study staff, study monitors, auditors and inspectors under confidentiality agreements.
The Sponsor provides all study documents to the Investigators and his/her appointed staff in confidence. Materials may not be disclosed to any party not involved in the study, unless written permission from the Sponsor or ITMs Data Access Committee with regard to the sharing of data for secondary research.

12.5 Risks and benefits

No major risks are anticipated for the participants. As outlined in the ethics section it is possible that when the study participant proposes to a partner that they both use the mouthwash product before sex that this may have an adverse effect on the encounter. It may even lead to the sex not taking place. In the informed consent process we will make it clear that if participants find that the use of the mouthwash pre and post sex has an adverse effect on their sex lives in any way then they may stop using the mouthwash peri-sex but we will ask that they continue with the daily mouthwash. Participation in the study will require no additional study visits but it will require the participant to spend slightly longer at each visit and in addition participants will be required to fill in the weekly diary.

Potential benefits of the study include the following:

- By lowering the risk of acquisition/transmission of STIs the participants can reduce the risk of antimicrobial exposure (for treating STIs) and symptomatic STIs to themselves and their partners.
- Raising awareness about the risk of STIs and in particular the role of oral sex in the transmission of STIs may lead to reduced risk taking

12.6 Compensation for participation

There will be no compensation for the study participation.

12.7 Insurance

As required by the Belgian law on experiments on the human person of May 7th 2004, the sponsor will obtain a no-fault liability insurance (with Amlin Insurance S.E.) covering any harm, injury or (material) damage which may occur to study participants and which may be directly or indirectly caused by their participation in the trial. The insurance provisions will also be mentioned during the informed consent discussion and in the informed consent form.

13. DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY

All study documents are provided by the Sponsor to the Investigators and his/her appointed staff in confidence. None of this material may be disclosed to any party not directly involved with the study, without written permission from the Sponsor.

Reporting and publication of the study data will be done in accordance with the CONSORT statement (check http://www.consort-statement.org/consort-statement/) and ITM's publication policy.

14. ARCHIVING

The sponsor and Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be verified. The relevant (essential) documents are those documents which individually and collectively permit to assess the conduct of the trial, the quality of the data produced and the compliance with GCP standards and applicable regulatory requirements. The Investigator's File should at least contain all the (essential) documents as listed in the procedure "Set up and maintenance of the Investigator Trial File". A copy of all source data and Case Report Forms must always be kept on site.

All the relevant study documentation should be retained for a minimum of twenty (20) years after completion of the study, as set out by the current Belgian law. The Sponsor should be informed prior to destruction of the files.
After completion of the study, the IF will remain available for internal audits and/or inspections of regulatory authorities for a period of twenty-five years.

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16. LIST OF ABBREVIATIONS

CASI	Computer assisted Structured Interview
CI	Coordinating Investigator
CLKB	Centraal Laboratorium Klinische Biologie
Ct	Chlamydia trachomatis
DSMB	Data and Safety Monitoring Board
(e-)CRF	(electronic) Case Report Form
ECG	Electrocardiogram
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GDPR	General Data Protection Regulation
IC(F)	Informed Consent (Form)
ICH	International Conference on Harmonization
(I)EC	(Independent) Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITM	Institute of Tropical Medicine
LCM	Listerine cool mint
Ng	Neisseria gonorrhoeae
PI	Principal Investigator
PrEP	Pre-Exposure Prophylaxis
QA	Quality Assurance
QC	Quality Control
RCT	Randomized controlled trial
(S)AE	(Serious) Adverse Event
SAP	Statistical Analysis Plan
STI	Sexually transmitted infection
SDV	Source Data Verification
SOP	Standard Operating Procedure
TMG	Trial Management Group
UZA	Universitair Ziekenhuis Antwerpen (~ University Hospital Antwerp)
WHO	World Health Organization

17. ANNEXES

Annex 1: Overview of strategy to characterize microbiome/resistomes

MIC

All strains will be tested for MICs using gradient Etest strips (AB Biodisk, Stockholm, Sweden)

Whole genome sequencing

The strains will be sequenced using both long (Pacbio Sequel ™) and short read technology (Illumina Inc). Genomic DNA will be isolated using Qiagen® MagAttract® HMW kit (qiagen) according to the manufacturer's protocol. Isolated genomic DNA will be sheared using Covaris G-tubes to fragment size distributions around 8-20kb, and barcoded SMRT bell libraries will be established following the Pacific Biosciences protocols. Libraries will be pooled at equimolar amounts, subsequently annealed with SMRT Sequencing Primer v3 and complexed with Sequel polymerase version 3.0. The DNA/Polymerase complex will further have purified using a Chromaspin TE400 column (ClonTech), and 2.5 fmol complexes will be loaded by onto a Sequel v3 SMRT cell, sequenced using Sequel v3.0 chemistry (10 h collection, diffusion loading), and results demultiplexed using Pacific Biosciences' software suite SMRTLink 6.0. For Illumina sequencing, high purity genomic DNA will be used to construct a tagmentation library by using Nextera XT DNA Sample Preparation Kit (Illumina, Inc). The library will be normalized and sequenced using Miseq Kit V3 (Illumina, Inc). The generated long reads, and short reads will be assembled using technology specific assemblies and hybrid assemblies, using SPAdes v3.13.0 and molecular characterization will be done using our automated in-house developed WGS pipeline Bacpipe v1.3 (Xavier et al, In preparation, 2018).

Microbiome/Resistome analysis: The metagenomic DNA samples will be prepared from the rectal and pharyngeal swabs from different time points (0, 3 months and 6 months) for shot-gun metagenome sequencing for microbiome/resistome analysis. DNA concentrations will be measured using NanoDrop (Thermo Scientific, Waltham, MA, USA) and Qubit (dsDNA assay).

Shotgun metagenomics bioinformatic analysis

The sample preparation and library preparations will be done using Nextera DNA Flex kit and sequencing using HiSeq2500 with v4 high output mode generating approximately 10-20 million reads of 100 bp length per sample.

Pre-processing pipeline: First, reads quality are checked using Fastqc. Next, reads are demultiplexed (Trim Galore), dereplicated (merging reads using DUST) and decontaminated via removing reads of non-intended genomes (e.g. human) using bowtie2. The reads are assembled into contigs *de novo* based using IDBA-UD. The produced assemblies would be visualised to validate its quality. Next, the contigs would be binned into individual genomes via MetaCluster tool. This binning would be quality checked via the core gene screening.

Next, the genomes would be annotated to identify coding regions via FragGeneScan. The functional properties of the coding regions, for each genome associated with Ng/Nf flora, would be assigned using BLAST2GO-Pro (searching KEGG/GO database). Additional analysis would be performed for resistance genes.

Post-processing and data visualization: For taxonomical classification, bar plots and calculate PCoA/nmds plots will be produced, this will help to provide an overview of the temporal shifts, core species associated with Ng/Nf commensal flora. For calculations of the relative abundance of antibiotic resistance genes, reads will be mapped against the CARD database https://card.mcmaster.ca/ using DIAMOND in blastx mode. Similarly, genes will be identified with LCM specific mutations in in *vitro* generated (Morbidostat) strains would be quantified by a total number of reads in the shot gun metagenomic data. It would also be possible to provide incite not only for bacterial taxonomy but also for fungal and viral species association.

Annex 2: Placebo Manufacture and Ingredients

The placebo will be manufactured by the designated pharmacy in line with regulatory guidelines. Both LCM and the corresponding placebo will be (re)packaged in identical containers and labeled with a study specific label which mentions the study name, the volume and expiration date and randomization number, according regulatory guidelines (box 2). The placebo has been tested in the STI Laboratory, ITM and found not to be inhibitory to the growth of N. *gonorrhoeae.*

Box 1: Placebo Ingredients (per 200ml)

- Sorbitol 30g,
- Sodium saccharin 0.1g,
- Solution Vitris Nf5 15 drops
- Aq Conservans Nf4 Ad 200g

Box 2: IMP label

FOR CLINICAL TRIAL USE ONLY

Study : PREGO

Sponsor : Institute of Tropical Medicine Antwerp

Expiration Date: MM/YYYY

Volume: ___ mL

RANDOMIZATION NUMBER : __ __ __ - __