Gono-Screen Study

Does screening for gonorrhea and chlamydia affect the incidence of these infections in men who have sex with men taking HIV pre exposure prophylaxis (PrEP): a randomized, multicentre controlled trial

GonoScreen: Efficacy of screening STIs in MSM

Version 2.1 dd 28/Apr/2021

ClinicalTrials.gov Number:	NCT04269434
KCE Trials Number:	INV18-1133
ITM Clinical Trial Number:	ITM202002

TABLE OF CONTENTS

■ SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Coordinating Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in "Directive 2001/20/EC" and any subsequent amendments, GCP guidelines, the Belgian law of May 7th 2004 regarding experiments on the human person, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:		
Signature:		Date: ...../...../.....
Name: Dr. Lut Lynen		
Position: Head of Department, Clinical Sciences ITM		
Coordinating Investigator and Principal Investigator Institute of Tropical Medicine (ITM):		
Signature:		Date: ...../...../.....
Name: Dr. Chris Kenyon		
Position: HIV/STI Clinician ITM		
Statistician:		
Signature:		Date: ...../...../.....
Name: Jozefien Buyze		
Position: ITM study biostatistician		
Principal Investigator Erasmus Hospital		
Signature:		Date: ...../...../.....
Name: Dr Sophie Henrard		
Position: EH HIV/STI Clinician		
Principal Investigator St. Pierre Hospital		
Signature:		Date: ...../...../.....
Name: Dr Agnes Libois		
Position: SP HIV/STI Clinician		
Principal Investigator U.Z. Gent		
Signature:		Date: ...../...../.....
Name: Dr Diana Huis in 't Veld		
Position: UZG HIV/STI Clinician		

Principal Investigator CHU Liège		
Signature:		Date: ...../...../.....
Name: Dr Anne-Sophie Sauvage		
Position: CHU HIV/STI Clinician		
For acknowledgement on behalf of the funder (KCE):		
Signature:		Date: ...../...../.....
Name (please print):		
Position:		

KEY TRIAL CONTACTS

Coordinating Investigator + Principal Investigator ITM	Dr. Chris Kenyon Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium ckenyon@itg.be 00 32 3 247 07 86
Trial Co-ordinator	Natacha Herssens/ Annelies De Hondt Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium nherssens@itg.be / adehondt@itg.be 0032 3 247 07 78 / 0032 3 247 07 16
Sponsor	Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen Belgium
Funder(s)	Belgian Health Care Knowledge Centre (KCE) Administrative Centre Botanique, Doorbuilding, Boulevard du Jardin Botanique 55, B-1000 Brussels, Belgium Trials@kce.fgov.be 00 32 2 287 33 88
Clinical Trials Unit	Yven Van Herrewege Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium yvanherrewege@itg.be 00 32 3 345 55 57
Statistician	Jozefien Buyze Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium jbuyze@itg.be 0032 3 247 64 93
Social science researcher	Thijs Reyniers Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium treyniers@itg.be 0032 3 247 65 39
Data Manager	Christophe Burm Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium

	cburm@itg.be 0032 3 247 64 55
Data Reviewer	Hanne Landuyt Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium hlanduyt@itg.be 0032 3 247 64 95
Committees	TSC: Dr. Chris Kenyon Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium ckenyon@itg.be TMG: Dr. Chris Kenyon Instituut voor Tropische Geneeskunde Nationalestraat 155, 2000 Antwerpen, Belgium ckenyon@itg.be
Principal Investigator Erasmus Hospital	Sophie Henrard Sophie.Henrard@erasme.ulb.ac.be Route de Lennik 808 B- 1070 Brussels
Principal Investigator St. Pierre Hospital	Agnes Libois Agnes_LIBOIS@stpierre-bru.be Department of Infectious Diseases. St Pierre University Hospital 322, rue Haute. B- 1000 Brussels
Principal Investigator UZ Gent	Diana Huis in 't Veld diana.huisintveld@uzgent.be Corneel Heymanslaan 10, 9000 Gent
Principal Investigator CHU Liège	Anne-Sophie Sauvage as.sauvage@chuliege.be Avenue de L'Hòpital 1, 4000 Liège

TRIAL SUMMARY

Trial Title	Does screening for gonorrhea and chlamydia affect the incidence of these infections in men who have sex with men taking HIV pre exposure prophylaxis (PrEP): a randomized, multicentre controlled trial
Short title	GonoScreen: Efficacy of screening STIs in MSM
Trial Design	Multicentre, controlled, randomized trial of 3 site (urethra, pharynx and rectum) sampling performed every 3 months (3x3) for *Neisseria gonorrhoea* (Ng)/*Chlamydia trachomatis* (Ct) screening (comparator) vs. no screening (intervention)
Trial Participants and setting	PrEP cohorts situated at the Institute of Tropical Medicine (ITM), Hôpital Saint-Pierre (HSP), University Hospital of Gent (UZG), Erasmus Hospital (EH) and Liège University Hospital (CHU). All men in follow up at these five centres who report having had sex with another man in the previous year and are enrolled for PrEP follow up will be eligible to participate in the study.
Intervention	Participants in the intervention arm will ***not*** be screened for Ng/Ct for a period of 12 months, in reference to the routine practice of screening (and treating screening-positive) participants every 3 months. More specifically, participants in the intervention arm will have 3 site (urethra, pharynx and rectum) sampling performed every 3 months but these testing results will only be reported at the end of the 12 month period.
Control	For a period of 12 months, all participants will be screened for Ng/Ct at three sites (urethra, pharynx and rectum) every three months according to current Belgian guidelines. If they test positive, they will be recalled for treatment for Ng/Ct and contact tracing.
Primary Endpoint	Incidence rate of Ng plus Ct detected at any site whilst individuals are screened vs. not screened.
Secondary Endpoint(s)	- Cumulative antimicrobial exposure (ceftriaxone/azithromycin/doxycycline) - Incidence of symptomatic Ng plus Ct - Incidence of syphilis - Incidence of HIV - Cost effectiveness of 3x3 screening vs. no screening - Variations in PrEP users' perceptions towards STI screening (only for ITM subjects)
Planned Sample Size	1014 participants
Intervention duration	12 months
Follow up duration	Not applicable
Duration of the trial (FPI-CSR)	2 years and 2 months (26 months)

FUNDING AND SUPPORT IN KIND

There are no other commercial organisations/entities involved in this study besides the KCE as a funder.

ROLE OF STUDY SPONSOR AND FUNDER

The Institute of Tropical Medicine as mentioned in KEY TRIAL CONTACT shall act as sponsor of the Study, as defined in the Law of 2004, and shall assume all responsibilities and liabilities in connection therewith and procure the mandatory liability insurance coverage in accordance with the Law of 2004. **The Institute of Tropical Medicine** shall ensure that it shall be mentioned in the Protocol, the Informed Consent Forms and in other relevant communication with the Study Subjects or the Regulatory Authorities as sponsor of the Study. **The Institute of Tropical Medicine** acknowledges and agrees for the avoidance of doubt that KCE shall under no circumstances be considered as sponsor of the Study or assume any responsibilities or liabilities in connection therewith, and **The Institute of Tropical Medicine** shall make no representations whatsoever in this respect.

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES

The GonoScreen Trial organogram is depicted in Figure 1.

1) Trial Steering Committee (TSC)

The Trial Steering Committee (TSC) will be responsible for the overall supervision and safety monitoring of the trial. The TSC will be constituted by the Principal Investigators (PIs) of the five study sites, the study statistician and a member of the ITMs Clinical Trials Unit (CTU). It will also include a representative from the participant community and a representative from the KCE who is independent of the investigators, their employing organisations and sponsors. The TSC will monitor trial progress, and give advice on the scientific implementation of the trial and it's continuation. The TSC is also responsible for the development of a study publication plan. The TSC will meet at least 3 times per year during the first year and two times a year after that. A meeting report will be written after every TSC meeting and will be distributed to all members of the TSC and TMG.

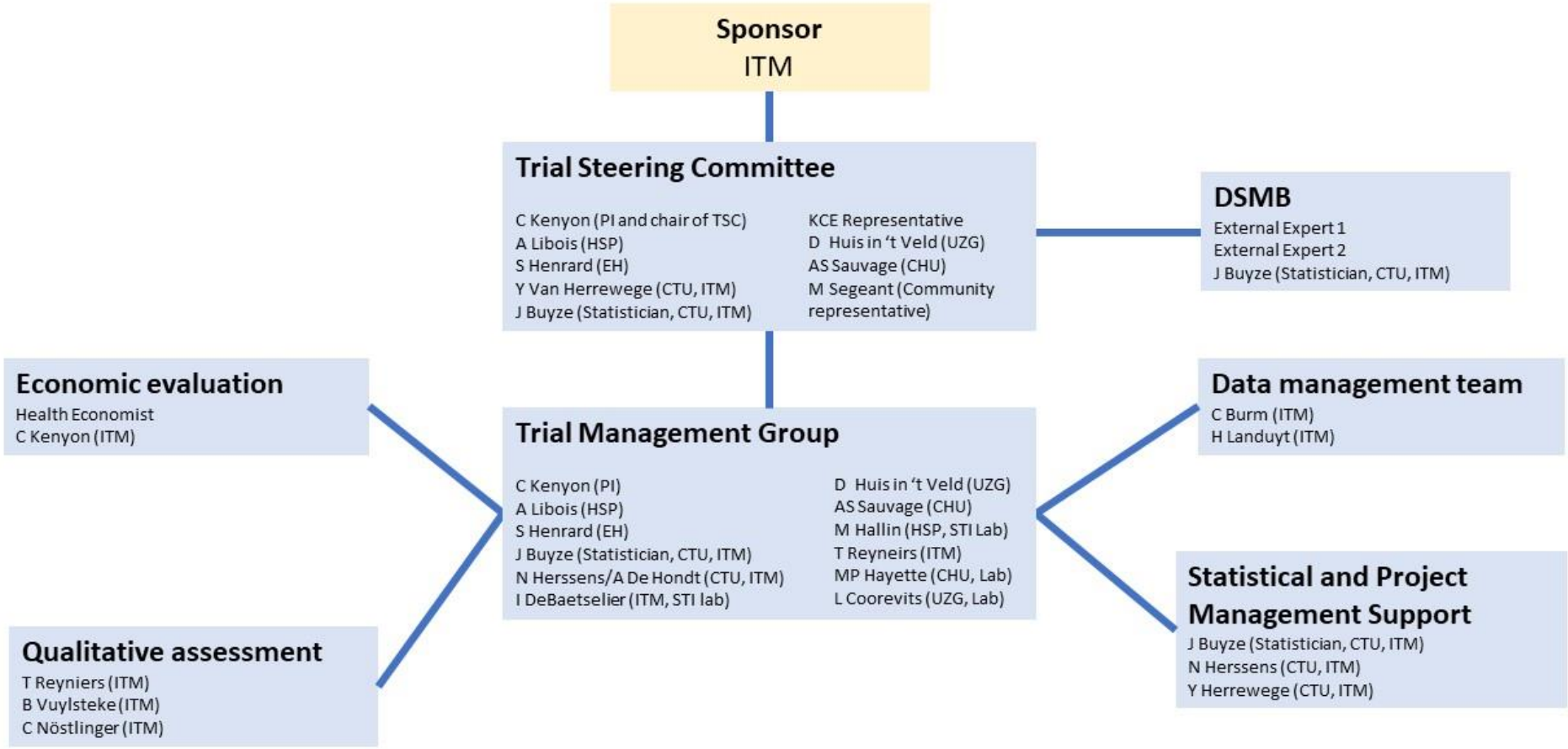
2) Trial Management Group (TMG)

The day-to-day management of the study will be performed by the Trial Management Group (TMG) which is distinct from the TSC. The TMG will consist of the same members as the TSC, except the representative from the participant community and the KCE representative will not be members. The following will be members: STI laboratory managers from ITM, HSP, EH, UZG and CHU and the head of the social science work package from ITM. The TMG will meet once a month on average during the whole duration of the trial and will be responsible for the daily management of the trial and site-specific questions/issues. After every TMG meeting, a report will be written including pending actions assigned to specific parties which will be distributed to all partners in the project.

3) Data and Safety Monitoring Board (DSMB)

We will set up an independent Data and Safety Monitoring Board (DSMB) to evaluate if the non-screening arm has an unacceptably high incidence of symptomatic Ng/Ct. Two independent STI experts (Infectious Disease Physicians/Epidemiologists) will constitute the DSMB and will be joined by the sponsor study statistician for the DSMB meetings.
The DSMB will evaluate the incidence of symptomatic Ng + Ct in both arms once 50% and 100% of individuals have completed their 6 month visit. If the incidence of symptomatic Ng + Ct in the non-screening arm is more than two-fold higher than the screening arm at either of these time points, then serious consideration should be given to stopping the study (further details in Section 10 and Appendix 1).

Figure 1. GonoScreen Trial Organogram



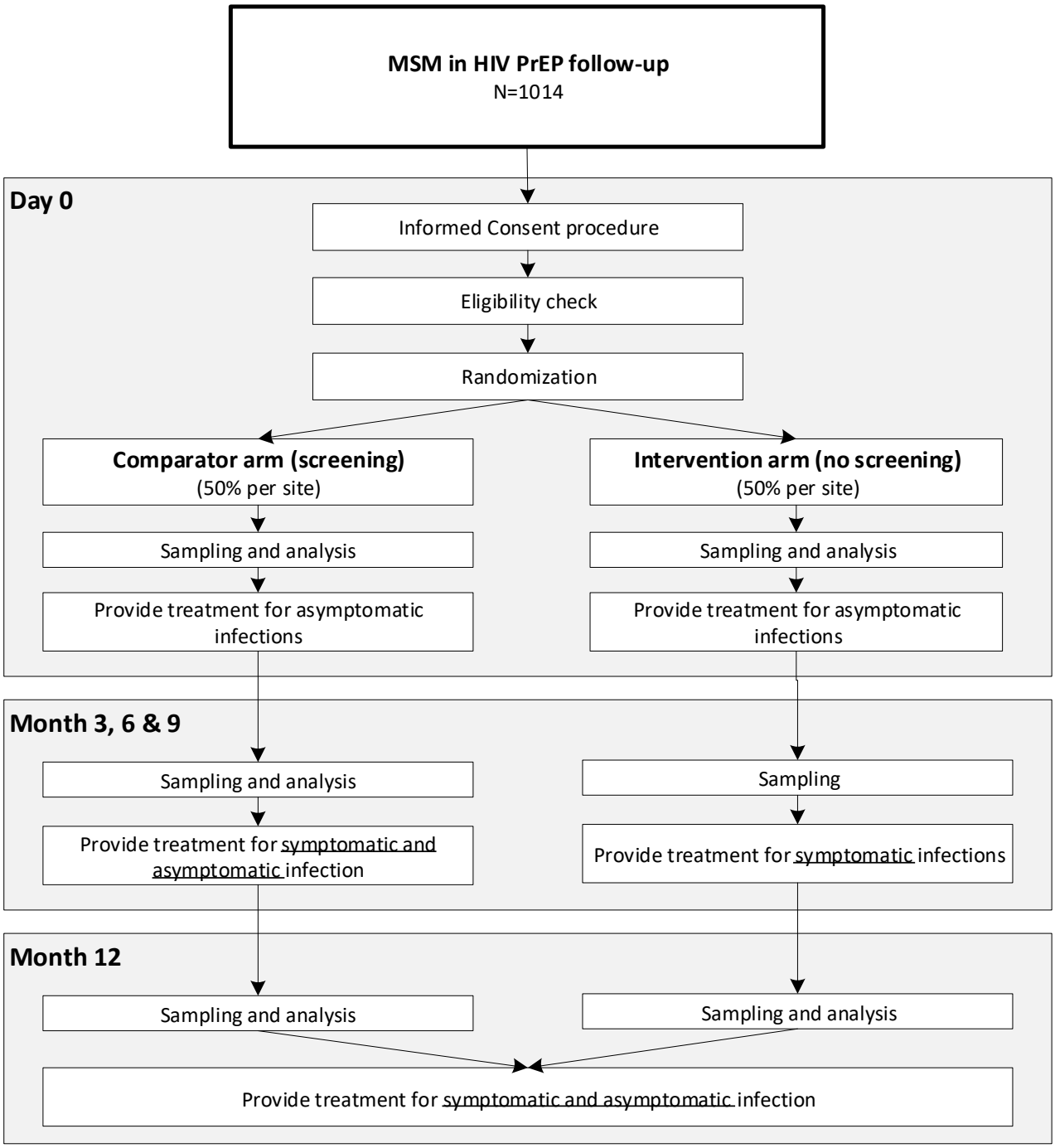
LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
AMR	Anti-Microbial Resistance
APR	Annual progress report
ARC	AIDS Reference Centre
BCFI	Belgian Centre for Pharmacotherapeutic Information
BREACH	Belgian Research on AIDS and HIV Consortium
CHU	Liège University Hospital
CI	Coordinating Investigator
CRF	Case Report Form
Ct	*Chlamydia trachomatis*
CTU	Clinical Trials Unit
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECDC	European Centre for Disease Prevention and Control
EH	Erasmus Hospital
EU	European Union
FAMHP	Federal Agency for Medicines and Health Products
FGD	Focus Group Discussion
FU	Follow Up
GCP	Good Clinical Practice
GCLP	Good clinical laboratory practice
GDPR	General Data Protection Regulation
GP	General practitioner
HIV	Human Immunodeficiency Virus
HSP	Hôpital Saint-Pierre
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.
IF	Investigator File
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ISF	Investigator Site File
ITM	Institute of Tropical Medicine
IUSTI	International Union against Sexually Transmitted Infections
KCE	Belgian Healthcare Knowledge Centre
LHUB	Laboratoire Hospitalier Universitaire de Bruxelles
MSM	Men who have Sex with Men
NAAT	Nucleic acid amplification testing
Ng	*Neisseria gonorrhoea*
PI	Principal Investigator
PIS	Participant Information Sheet

PrEP	Pre-Exposure Prophylaxis
QA	Quality Assurance
QoL	Quality of life
RCT	Randomised Control Trial
RIZIV/INAMI	Rijksinstituut voor ziekte- en invaliditeitsverzekering/ Institut national d'assurance maladie-invalidité
SAP	Statistical analysis plan
SDV	Source Data Verification
SIV	Site initiation visit
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
TDF/FTC	Tenofovir disoproxil fumarate/Emtricitabine
TMG	Trial Management Group
TSC	Trial Steering Committee
TMF	Trial Master File
USA/U.S.	United States of America
USV	Unscheduled Visit
UZA	University Hospital of Antwerp
UZG	University Hospital of Gent
WHO	World Health Organization
3x3 screening	Screening 3-sites -pharynx, rectal and urethral- at 3 monthly intervals

TRIAL FLOW CHART

Figure 2. Trial flowchart



■ STUDY PROTOCOL

1 BACKGROUND

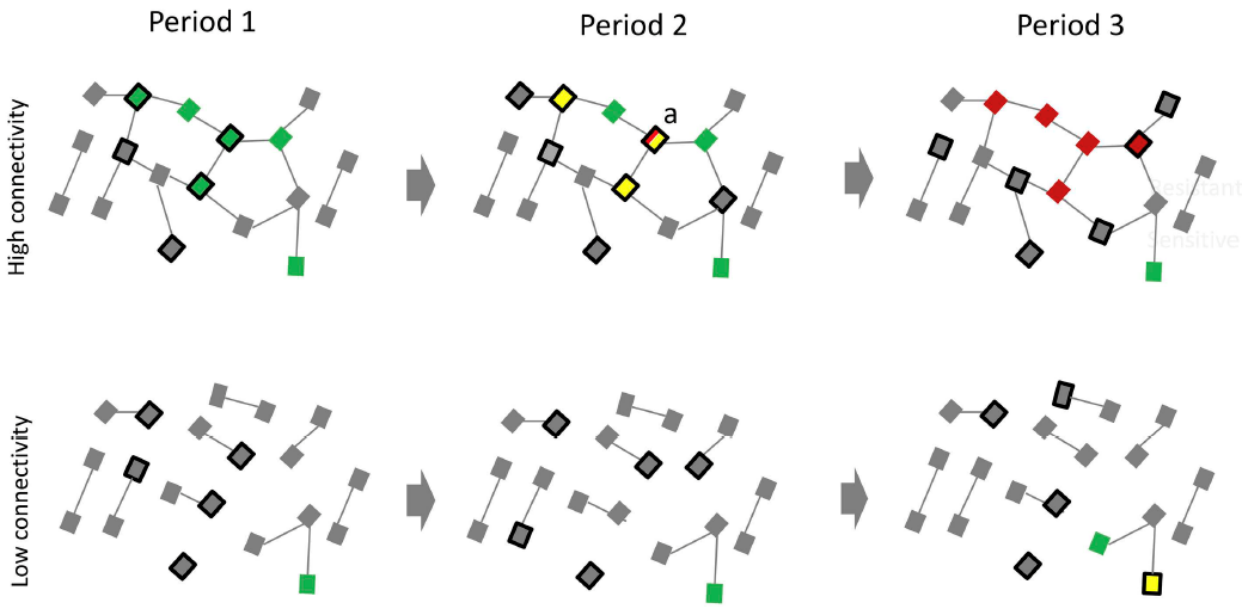
Neisseria gonorrhoeae (Ng) is a common sexually transmitted infection whose incidence has been noted to be increasing in key populations such as men who have sex with men (MSM) taking HIV pre exposure prophylaxis (PrEP) in many countries [1, 2]. In part, this increased incidence is an artefact of more intensive screening [3]. Longitudinal studies of MSM PrEP cohorts have typically found a high but stable incidence of Ng and *Chlamydia trachomatis* (Ct) [2]. Infections in MSM can occur at 3 sites – the oropharynx, urethra and anorectum [1, 2]. The vast majority of infections in MSM are asymptomatic and self-limiting [1]. The high incidence of Ng and Ct in MSM PrEP cohorts has prompted calls for frequent screening for these infections [4]. The Belgian PrEP guidelines, for example, currently mandate 3x3 screening (screening 3-sites -pharynx, rectal and urethral- at 3 monthly intervals) for all PrEP recipients [2, 5]. Of note these guidelines were not based on evidence from clinical trials but based on the fact that guidelines from other countries recommend 3x3 screening, even in the absence of scientific data from clinical studies in these countries [2, 5]. Because there is so little evidence to justify screening and screening entails an appreciable cost, there is considerable heterogeneity in the intensity of Ng/Ct screening between PrEP centres within Belgium. In some centres, only one site is tested every 3-6 months. In other centres all three sites are tested every 3 or 6 months.

Possible negative effects of intensive screening

A major concern of this intensive screening is that it will select for antimicrobial resistance (AMR) in Ng and other bacteria. Because the vast majority of Ng and Ct are asymptomatic in MSM, 3x3 screening and subsequent treatment (compared to only treating symptomatic infections) increases antibiotic exposure by up to 60-fold [6] and results in consumption rates of azithromycin and ceftriaxone that have been found to result in high rates of AMR in a range of bacteria in various studies [7-9]. One analysis for example found that 3x3 screening in PrEP cohorts results in macrolide exposures of up to 4400 standard doses/1000 population per year [6]. This exposure level is 41 times greater than that of populations such as Latvia and considerably higher than exposure levels found to be strongly associated with the induction of antimicrobial resistance (AMR) in a range of bacteria [6]. This degree of antimicrobial exposure carries a high risk of producing resistant microbiomes and thereby increasing the risk of infections with resistant bacteria. This includes both non-STIs (Sexually transmitted infection) such as *Staphylococcus aureus* as well as STIs such as Ng and *Treponema pallidum* (Figure 3). We and others have found evidence that populations with higher levels of antimicrobial consumption have higher rates of AMR in Ng [9-11]. Furthermore, we have found historical evidence that the intensive Ng screening and treatment campaign in Greenland in the 1960s was followed by a steep-increase in gonococcal AMR [12]. Finally, we have also found ecological level associations between the intensity of Ng/Ct screening in MSM and gonococcal AMR in both the USA and Europe [13, 14].

Figure 3. An illustration of how frequent screening for *N. gonorrhoeae* in MSM PrEP populations may have little effect on reducing prevalence of N. gonorrhoeae but result in the development of antimicrobial resistance.

Period (1) The high sexual network connectivity of a typical PrEP cohort (top) translates into a high equilibrium prevalence of N. gonorrhoeae (green squares). Period (2) Active screening of a quarter of this population (black bordered squares) results in a lower N. gonorrhoeae prevalence in period 2 but at the expense of an altered resistome (yellow squares represent individuals with N. gonorrhoeae cleared via antibiotics in preceding period). Because the network connectivity remains unchanged, N. gonorrhoeae tends to return to its equilibrium prevalence. This places recently cured individuals (such as individual "a") at high risk of reinfection at a time when their resistomes are enriched with resistance genes (azithromycin for example leads to elevations of resistance genes for up to 4 years [15]). An early N. gonorrhoeae reinfection in "a" is able to take up these resistance genes via transformation and become resistant to the antibiotics used to treat N. gonorrhoeae (red squares). Period (3) If there is ongoing high exposure to antibiotics these less susceptible N. gonorrhoeae strains will have a fitness advantage over more susceptible strains. These dynamics would be predicted to favor the emergence and spread of resistant N. gonorrhoeae. By period 3, N. gonorrhoeae has returned to its equilibrium prevalence for this degree of network connectivity but now most strains are resistant. The degree of connectivity in the low connectivity population (bottom) is so low that N. gonorrhoeae remains at a very low prevalence. Even extensive screening is unlikely to result in sufficient antibiotic exposure to provide N. gonorrhoeae access to resistance genes or a fitness advantage for resistance strains (Uninfected individuals: gray squares; Edges between squares represent sexual relationships) (Figure from [16]).



Preventing the emergence of untreatable gonorrhoea

Ng has developed AMR to every single antibiotic used against it [17, 18]. This plus recent reports of combined high level resistance to ceftriaxone and azithromycin have led to concerns it will be untreatable in the near future [17, 18]. The World Health Organization and others classify Ng as being at high risk for further development of AMR and one of the strategies advocated to reduce

the probability of further AMR developing is antimicrobial stewardship [19, 20]. This involves reducing the use of antimicrobials to clearly defined indications where benefit clearly outweighs risk [20, 21]. Gonococcal AMR has typically emerged in core groups such as MSM PrEP cohorts that have high rates of partner change and high levels of antimicrobial consumption [22, 23]. These considerations mean that it is critical to ensure that antimicrobial consumption in MSM PrEP cohorts is limited to indications where there is clear evidence of benefit [6, 16].

Which STIs should be screened for in MSM?

According to the World Health Organization and others, screening programs should only be introduced if they fulfill various criteria [24]. Two of these criteria are that there should be scientific evidence of screening effectiveness and that the overall benefits of screening should outweigh the harms [24].

The increasing concerns about AMR and reducing excessive antibiotic consumption in MSM have led us and others to re-evaluate the available evidence as to the benefits and harms of screening for each STI in MSM [4, 16, 22]. We concluded that whilst the evidence in favour of screening was strong for HIV, syphilis and hepatitis C, it was weak and contradictory for Ng and Ct [16]. These conclusions were based on the following types of evidence:

- ***No Randomised Control Trial (RCT) have ever been conducted*** or are planned in MSM or other men to evaluate the benefit/risk of Ng/Ct screening [5]. As a result, the U.S. Preventive Services Task Force guideline concludes that there is insufficient evidence to make a recommendation as to whether or not screening for these infections should be conducted in men [5].
- A ***systematic review of observational studies*** in MSM found that screening was not associated with reductions in prevalence of Ng or Ct [6]. Evidence from the ITM's PrEP cohort, for example, demonstrates that the prevalence of Ng and Ct remain about 10% each despite 3 monthly screening/treatment [7].
- We have evaluated if there was an ***ecological association between screening intensity and Ng prevalence*** in MSM. There are wide variations in the intensity of self-reported Ng/Ct screening in MSM both between countries within Europe and within States in the United States of America. In neither of these examples is there a *negative* association between screening intensity and Ct or Ng prevalence in MSM. For example, in Europe we compared self-reported national STI screening rates from a behavioural survey of 180 000 MSM from across Europe (European MSM Internet Survey) with European Centres for Disease Prevention and Control national estimates of Ng and Ct incidence from the same year 2010 [25]. We found *positive* association between these variables for both Ng and Ct (Rho =0.74; P=0.0004 and Rho=0.71; P=0.001, respectively). This positive association may be due to the fact that more intense Ng/Ct screening would be expected to generate higher incidence estimates. To avoid this bias, we used incidence estimates generated from more comparable studies of Ng/Ct prevalence estimates from MSM attending STI clinics. We found no evidence of an association between screening intensity and Ng incidence [25]. The results from the USA were similar (Unpublished data).
- ***Modelling studies*** have reached somewhat different conclusions as to the efficacy of Ng/Ct screening [8]. One study found that screening may lead to meaningful reductions in Ng prevalence [26]. A modelling study by our group that, unlike the prior study, included

pharyngeal transmission found screening to have a minimal effect on Ng prevalence in Belgian MSM but a large effect on antimicrobial exposure [3].

- ***The biology of Ng and Ct host interactions make them less amenable to screening than HIV, syphilis and hepatitis C*** [16]. In the case of Ng, numerous aspects of the way it circulates in MSM decrease the probability that screening will be beneficial. Symptomatic disease is thought to typically occur soon (2–21 days) after infection. If symptoms do not develop, the infection (particularly in the pharynx and rectum) tends to persist in a low abundance state for one to 6 months [27]. Highly exposed individuals develop a type-specific immunity but this immunity is largely ineffective in low exposure individuals [27, 28]. The vast majority of Ng infections are asymptomatic and self-limiting in this population [27, 28]. Screening is far more likely to diagnose infections in the 6 month asymptomatic tail phase (when Ng abundance is likely lower and therefore less infectious) than in the acute first weeks post infection. These features reduce the probability that screening will decrease either symptomatic infections or Ng transmissions—assuming that the low abundance infections are less infectious. Similar considerations apply to Ct. In the case of C. trachomatis there is however better evidence that treatment of Ct results in "arrested immunity" and thereby paradoxically increases the probability of reinfection and may even lead to increases in prevalence [29, 30].
- ***High risk of inducing AMR***. As noted above, gonorrhea/chlamydia screening in MSM can result in exposure levels to macrolides/cephalosporins that are strongly associated with resistance in Ng, *Treponema pallidum* and a range of other bacteria [8-10].

The fact that we have no clear evidence of 3x3 Ng/Ct screening being beneficial in this population combined with the fact that this policy results in such high rates of antimicrobial consumption in this population mean that randomized controlled trials are urgently required to assess the utility of 3x3 screening [16].

2 RATIONALE

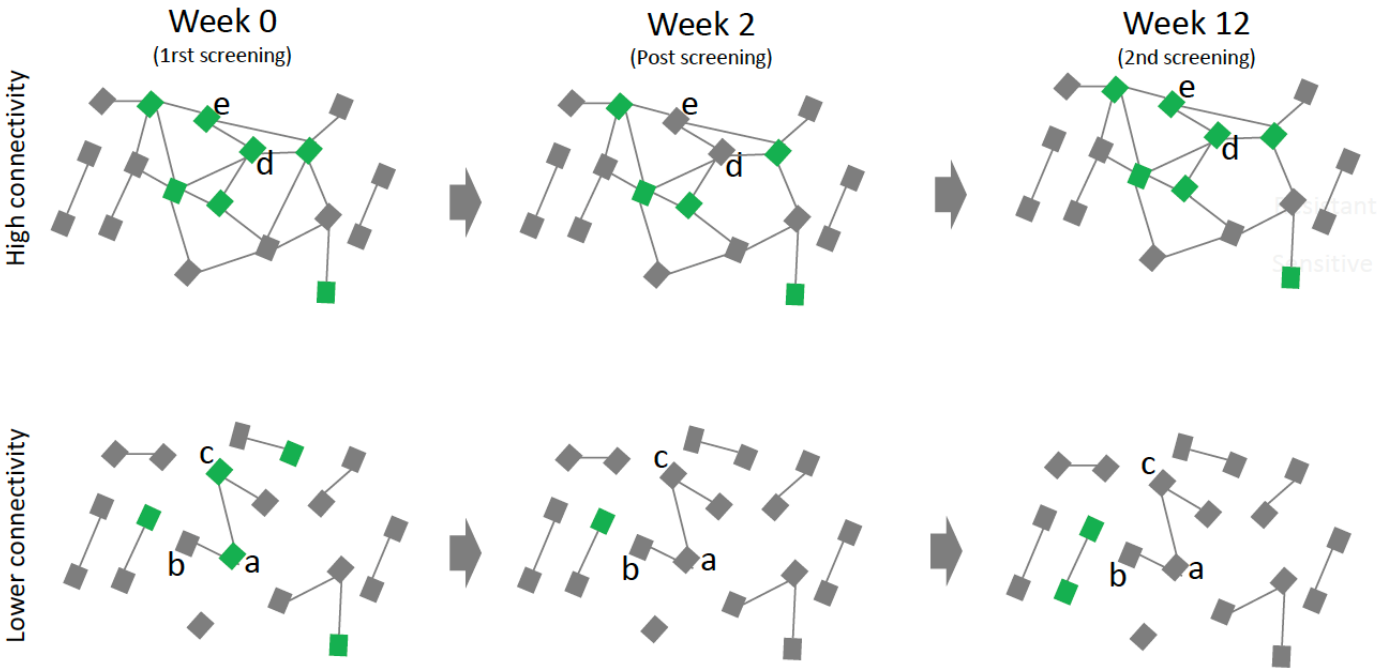
Our randomized controlled trial aims to establish if screening results in a clinically meaningful and cost-effective reduction in Ng/Ct incidence in MSM PrEP cohorts that could outweigh the increased risk of AMR development it confers. We considered conducting a cluster randomized controlled trial to evaluate if screening is associated with a reduction in Ng/Ct prevalence at population level. The cost of such a study would be very high [31] and evidence from behavioural surveys and HIV/STI phylogeny in Belgian MSM indicates a very high rate of sexual mixing between populations in different Belgian towns [32-35]. As a result, the probability of effect-contamination between randomized centres would be high. These factors are not unique to Belgium and consequently a number of authors have concluded that this type of community level RCT is unlikely to ever take place [23, 31].

Furthermore, as already noted, the available evidence from ecological analyses, our systematic review of observational data of the effect of Ng/Ct screening on prevalence as well as evidence from modelling evaluations all suggest that screening is unlikely to have a noticeable effect on the prevalence of Ng/Ct at a population level in contemporary PrEP cohorts.

This still leaves the important clinical question of whether or not screening at an individual level is able to reduce the risk of acquiring Ng and Ct. Even if 3x3 screening has no effect on Ng/Ct prevalence at a population level, it could reduce prevalence at an individual level. This is illustrated in Figure 4 which contrasts schematically the impact of Ng screening on two populations – one with a densely connected sexual network and one with a somewhat lower network connectivity. Starting with the lower connectivity population (lower panel), if 8% of the network is screened weekly (corresponding to 100% coverage with 3 monthly screening) and this includes individual 'a', then screening in this setting is likely to reduce the probability that ‘a’ will be infected with Ng (green squares) by the time of his second screening (week 12) via two mechanisms. Firstly, screening will result in ‘a’s Ng being detected and eliminated which will prevent it spreading to 'a's uninfected partner (‘b’) which will in turn reduce the probability that 'a' will be infected with Ng at his next visit. Secondly, if 'a' is found to test positive for Ng then his partners will be invited for testing which could result in his Ng-infected-partner (‘c’) being treated for Ng which would further reduce the probability that 'a' would be infected with Ng at subsequent visits.

Alternatively, the local sexual network could be so dense (upper panel) that 3x3 screening may have little or no impact on prevalence. In this scenario, the Ng from ‘d’ and one of his infected partners (‘e’) is eliminated by screening at baseline but his local sexual network is so dense that he is rapidly re-infected with Ng. Screening in this setting has little probability of reducing the probability of incident Ng infection for individual ‘d’ or others in this sexual network.

Figure 4. An illustration of how Ng screening in a lower connectivity sexual network may reduce the probability of incident Ng for and individual 'a', whereas it has little or no effect in a high connectivity network. See text for details. (Ng infected individuals- green squares; Uninfected individuals- grey squares; Edges between squares represent sexual relationships).



These considerations have resulted in the current proposal where we plan to conduct an individual level RCT to assess if 3x3 screening for Ng/Ct is associated with a reduced incidence of these infections after 12 months screening versus no screening. One of the secondary outcomes will be assessing the efficacy of screening in those with relatively lower risk behavior (defined as 4 or fewer partners per 3 month period; represented by the lower connectivity network in Figure 3).

We are aware that participants in the no-screening arm would not be receiving the standard of care prescribed according to the Belgian PrEP guidelines. These PrEP guidelines were however developed by the Belgian Research on AIDS and HIV Consortium (BREACH) and discussions within BREACH informed by local research have led to serious doubts about the merit of 3x3 Ng/Ct screening [4, 6, 12, 14, 25]. For the reasons outlined above, we are concerned that our current guidelines may even be doing more harm than good. As a result, the 2018 annual general meeting of BREACH endorsed an RCT to evaluate the benefits and harms of 3x3 screening in Belgium PrEP cohorts. This study is thus the product of local efforts to produce evidence to help establish what the best standard of care should be vis-à-vis screening for Ng and Ct in PrEP recipients.

Phase II - cessation of Ng/CT screening in MSM

If the GonoScreen Study finds that screening is not associated with a reduced incidence of Ng plus Ct infections, then a second phase of the study is foreseen (separate from the current study proposal). In this second phase, we will present the GonoScreen Study results to BREACH with the recommendation that we cease screening MSM (PrEP recipients and other MSM) whilst closely monitoring the impact this has on incident STIs in a yet to be agreed upon strategy.

3 ASSESSMENT AND MANAGEMENT OF RISK

We acknowledge the ethical concern that the study may result in an increase in symptomatic disease in individuals (and their partners) in the no-screening arm. We justify this possible increased risk by an appraisal of the available evidence which reveals that we have no high-quality evidence that the 3-monthly, 3-site Ng/Ct screening in MSM PrEP cohorts (3x3 screening) is beneficial. We do have evidence that 3x3 screening likely results in harm [6, 16]:

1. **Adverse effects of antibiotics** as noted above[15, 36-39].
2. **Arrested immunity** as noted above [29, 30]. If screening results in 'arrested immunity' it may thus paradoxically increase Ng/Ct prevalence/symptomatic disease.
3. **Certificate of health effect.** A number of studies have argued that screening may result in a "certificate of health" effect whereby those screened may feel that if they come for regular screening this gives them a bill of health that means they can relax safety devices perceived to be onerous such as condom use [16, 40].

Our risk assessment concludes that based on the available evidence stopping screening may be associated with either an increase or a decrease (given arrested immunity) in symptomatic Ng/Ct infections and Ng/Ct spread. Stopping screening will almost certainly be associated with a lower antimicrobial exposure and lower costs.

To minimize the risks, the study is planned to be conducted in a limited and closely-monitored cohort of MSM, already attending scheduled visits at the 5 HIV PrEP clinics. As such, participants will continue to receive appropriate counselling related to practicing safe sex. Participants in the intervention arm will be followed-up with the same intensity and frequency as participants in the comparator arm. In both arms, symptomatic infections, including contact tracing, will be treated throughout the study period and according to standard of care. The safety monitoring board will evaluate if there is an increase in the incidence of symptomatic Ng/Ct infections at the 6 month visits in the non-screening arm. If this would exceed a pre-specified threshold, the study will be stopped (See Section 10).

4 OBJECTIVES AND ENDPOINTS / OUTCOME MEASURES

Study hypothesis

The study hypothesis is that 3x3 screening in MSM PrEP recipients is not associated with a reduction in the incidence of Ng plus Ct infections over 12 months.

Table 1. PICO Table

Population	**All MSM participants aged 18 or older, enrolled to receive HIV PrEP at the Institute of Tropical Medicine, Antwerp (ITM), Hôpital Saint-Pierre (HSP), University Hospital of Gent (UZG), Erasmus Hospital (EH) and Liège University Hospital (CHU) who consented to participate in the trial. Participants must report having had sex with another man in the prior 12 months.**
Intervention	Participants in the intervention arm will not be screened for Ng/Ct for a period of 12 months, in reference to the routine practice of screening (and treating screening-positive) participants every 3 months (see 'Comparator'). More specifically, participants in the intervention arm will have 3 site (urethra, pharynx and rectum) sampling performed every 3 months but these testing results will only be reported at the end of the 12 month period. Those testing Ng and/or Ct positive at their last visit in the 12 month period will be recalled and offered treatment even in the case of asymptomatic infection (cfr. current routine care). All individuals with symptomatic infections will be treated according to current best practice guidelines, including contact tracing. Participants in the intervention arm will also be treated if contacted via contact tracing.
Comparator	3x3 screening. For a period of 12 months, all participants will be screened for Ng/Ct at three sites (urethra, pharynx and rectum) every three months according to current Belgian guidelines. If they test positive they will be recalled for treatment for Ng/Ct and contact tracing.
Outcome	**Primary outcome:** Incidence rate of Ng plus Ct detected at any site whilst individuals are not screened vs. screened **Secondary outcomes:** • Cumulative antimicrobial exposure (ceftriaxone/azithromycin/ doxycycline) • Incidence of symptomatic Ng plus Ct • Incidence of syphilis • Incidence of HIV • Cost effectiveness of 3x3 screening vs. no screening • Variations in PrEP users' perceptions towards STI screening (only for ITM subjects)

4.1 Primary objective

To assess if not screening MSM on PrEP for Ng/Ct is non-inferior compared to screening in terms of the incidence rate of these infections over a 12-month period.

4.2 Secondary objectives

1) To compare the antimicrobial exposure (ceftriaxone/azithromycin/doxycycline) in the screening and non-screening arms
2) To assess if not screening MSM on PrEP for Ng/Ct is non-inferior compared to screening in terms of the incidence rate of symptomatic Ng and Ct infections
3) To assess the incidence of syphilis
4) To assess the incidence of HIV
5) To evaluate the economic impact of cessation of Ng/Ct screening in MSM
6) To explore PrEP users' perceptions towards STI screening (only for ITM subjects)

4.3 Endpoints

4.3.1 Primary endpoint

Incidence of Ng plus Ct detected at any site whilst individuals are screened vs. not screened.

Numerators: Cumulative number of diagnoses of Ng plus Ct in 12 months in screening/non-screening arms. Each participant can only contribute one diagnosis of Ct and one diagnosis of Ng per (scheduled or unscheduled) visit - regardless of number of sites infected. Thus each participant can contribute up to 2 diagnoses (Ct/Ng) at each (scheduled or unscheduled) visit. The numerator includes laboratory-confirmed diagnoses made between scheduled visits, performed inside or outside of the study.

Denominators: Number of scheduled study visits with available results for the diagnosis of Ng/Ct. The denominator does not include unscheduled visits[a].

The diagnosis of Ng and Ct will be made via nucleic acid amplification testing (NAAT) performed on rectal and pharyngeal swabs and urine.

In the primary analysis, the primary endpoint (as described above) includes all Ng/Ct diagnoses. Hence it is implicitly assumed that every diagnosis is a new infection. It is however possible that an Ng/Ct infection detected at the 3 to 12 month visit in the non-screening arm is simply a non-resolved infection from the prior visit (see Figure 5) [b]. This could spuriously increase the measured incidence in the non-screening group. Because

[a] The rationale for this definition of the denominator is to, as closely as possible, reflect time at risk of testing Ng/Ct positive. If an individual misses one of their 4 study follow up visits then then this reduces their probability of being diagnosed with Ng or Ct by one quarter (assuming no other relevant variables change). Our denominator definition takes this into account by only including study visits where we have results of Ng/Ct testing. Unscheduled visits are not included as these make up a very small proportion of all visits and could be considered to bias the time at risk estimate.

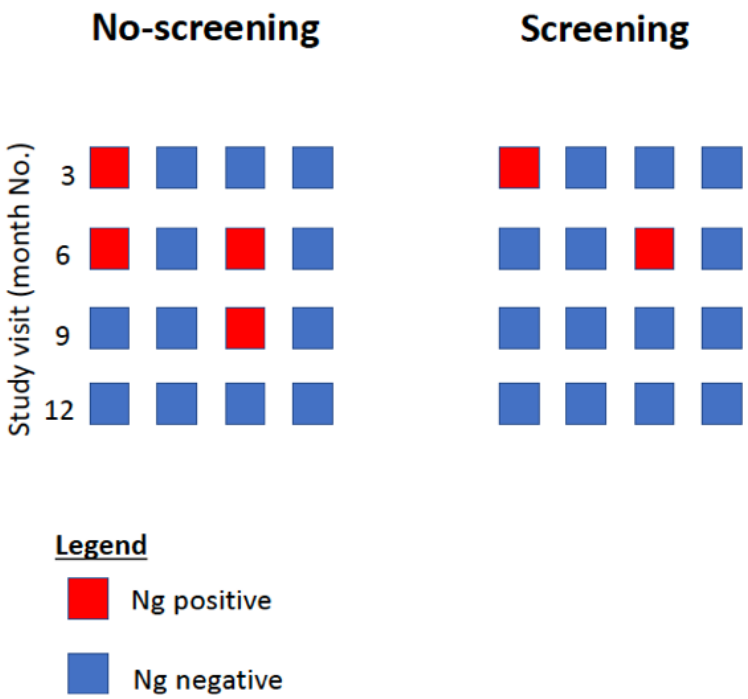
[b] Given the natural rate of clearance of Ng and Ct from each site, this probability is very low

this risk only applies to the non-screening arm it results in a bias towards exaggerating the effect of screening on incidence. As such if our study finds that non-screening is non-inferior to screening this finding could not be explained by this bias. In a sensitivity analysis, the numerator of the primary endpoint will only include diagnoses if there was a negative test result at the prior visit.

This is further illustrated for gonococcal infections in Figure 5, where over the 12 month follow up there are 4 incident Ng infections in the 4 individuals considered in the no-screening arm. These 4 incident infections are classified according to the study methodology. These 4 infections actually occurred in 2 individuals on 2 contiguous visits meaning it is possible (though very unlikely) that the second of each of these infections represent merely the end stages of the natural elimination of the first infection which still test positive on Ng Nucleic Acid Amplification Test (NAAT).

This would not occur in the screening arm where both these infections would be treated and eliminated at the visit at which they were first detected. The net effect would be a spurious high incidence of Ng in the no-screening arm. Similar considerations would apply to Ct. Because this bias exaggerates the effect of screening, if our study finds that no-screening is non-inferior to screening then this finding could not be explained by this bias.

Figure 5. The non-resolved infections bias



Test of cure: The studies approach to positive Ng/Ct test results at a test of cure visit is outlined in the 'Test of cure visit' section on page 30 (section 8.4).

4.3.2 Secondary endpoints

7) Cumulative antimicrobial exposure (ceftriaxone/azithromycin/doxycycline), measured as number of standard doses per 1000 person-years [41]
8) Incidence of symptomatic Ng plus Ct
9) Incidence of syphilis
10) Incidence of HIV
11) Economic impact of cessation of 3x3 screening in MSM in Belgium
12) Variations in PrEP users' perceptions towards STI screening (only for ITM subjects)

5 TRIAL DESIGN

This study is a multi-centre, controlled, randomized trial of 3x3 Ng/Ct screening (comparator) vs. no screening (intervention). It will be performed in the PrEP cohorts situated at the ITM, HSP, CHU, UZG and EH. All men in follow up at these five centres who report having had sex with another man in the previous year and are enrolled for PrEP follow up will be eligible to participate in the study. After signing informed consent participants will be randomized via a computer-generated schema to either 3x3 screening or no Ng/Ct screening (see section 8.3). In both arms, participants will be followed up in an identical fashion including 3x3 screening. The only difference between the arms will be that in the screening arm, Ng/Ct results will be sent by the STI Laboratory to the study physicians and these participants will be treated and partner contact tracing will be done. The STI Laboratory will only process the samples/report the results from the non-screening arm at the end of the study. In both arms, all individuals with symptoms compatible with Ng or Ct will be tested and treated for these infections according to current best practice guidelines. At the end of the 12-month study period, participants whose most recent tests were positive for Ng or Ct will receive treatment for these.

6 STUDY SETTING

This multi-centre trial will be performed in 5 different sites: Institute of Tropical Medicine, Antwerp (ITM), Hôpital Saint-Pierre (HSP), University Hospital of Gent (UZG), Erasmus Hospital (EH) and Liège University Hospital (CHU). All MSM participants aged 18 or older, enrolled to receive HIV PrEP at these 5 sites and who consented will be eligible to participate in the trial. Participants must report having had sex with another man in the prior 12 months. There are currently 803/816 /290/260/240 MSM enrolled in the ITM/HSP/EH/CHU/UZG PrEP cohorts.

7 ELIGIBILITY CRITERIA

7.1 Inclusion criteria

- Able and willing to provide informed consent
- Men (born as males) and transwomen aged 18 or more*
- Has had sex** with another man in the last 12 months

- Enrolled in Belgian PrEP program at ITM/HSP/EH/CHU/UZG with approval for TDF/FTC (Tenofovir disoproxil fumarate/Emtricitabine) reimbursement from a Belgian Medical Aid
- Willing to comply with the study procedures and to attend the clinic for the 3-monthly visits

7.2 Exclusion criteria

- Enrolment in another interventional trial
- Tests HIV-positive at screening
- Symptoms of proctitis or urethritis

* Cis-women and transmen will not be eligible to participate. This is because of the concern that undetected Ng /Ct infections may cause pelvic inflammatory disease. According to the literature, and our own experience, the numbers of transmen taking PrEP is very low. To the best of our knowledge, we do not have any transmen in follow up at the ITM PrEP cohort. The number of cis-women taking PrEP in our cohorts is also very low.
** Having had sex with another man is defined as having had peno-oral / peno-anal sex with another man in the last 12-month period.

8 TRIAL PROCEDURES

8.1 Recruitment

8.1.1 Participant identification

Potentially eligible participants will be identified by the attending PrEP doctors during their routine 3-monthly visits to the study site for their PrEP follow-up. A typical topic of discussion during PrEP consultations is what the PrEP recipients' preference is for STI screening. As part of these discussions the PrEP recipient will be informed about the GonoScreen Study and they will be asked if they wish to participate. It will be made clear to PrEP recipients that they are not under any pressure to participate in the study. In addition, they will be notified that they are free to leave the study at any point without any bearing on their ongoing PrEP care. The PrEP doctors at each of the 5 participating centres will thus be the only individuals who screen for eligible participants. These same PrEP doctors will also be responsible for confirming study eligibility and taking informed consent from the participants.

Posters and leaflets with information about the study will be available in the waiting room of the study site. These documents and materials will be submitted to and approved by the Ethics Committees (ECs) before use.

Participants at the ITM who declined to participate in the GonoScreen study will be asked whether the social science researcher(s) of this study may invite them for a brief qualitative study (see 8.10). Again, it will be made clear that they are not under any pressure and that they may decline at any time. We will explain that this study would be to explore their opinion towards STI screening, to which there is no correct or incorrect answer.

8.1.2 Screening

There are slightly different schemas for screening and starting PrEP in the 5 participating centres. What is most important for the study is that by 3 months after starting PrEP all centres follow their patients up every 3 months. In order to standardise the time periods used to compare incidence

we will only enrol participants from the point when they are on PrEP and their next follow up visit at a 3 monthly interval, which is typically at their first or second PrEP visit. Participants who are already taking part in the PrEP program will be also be eligible and can be enrolled.

There are two schemas used for lab testing:

- **Day-of-visit testing.** The tests for Ng/Ct and other laboratory tests are performed on the day of the clinic visit. The participant is typically contacted the following week in the event of an abnormal laboratory test for further treatment/intervention.
- **Week-prior testing.** Here the laboratory tests are performed the week prior to the clinic visit and the results are available to discuss during the clinic visit.

The results of these tests will be used for documentation purposes after the participant has given consent to participate. The test itself is to be considered as a standard of care screening test.

In the event of a positive test for Ng/Ct at this screening visit, they will receive appropriate therapy regardless of which arm they are assigned to.

If an individual has ***symptomatic*** urethritis or proctitis at the time of the study visit they will not be eligible for study enrolment at that visit. They will however be eligible for enrolment at one of their subsequent study visits if their symptoms have resolved by this time point.

Testing positive for syphilis and receiving treatment for syphilis is not an exclusion criteria for participation at the screening or any other visit.

Eligibility for study participation will be confirmed by the study doctors. Study participants will not receive any reimbursement for participation.

8.2 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. Translation(s) will be done using DeepL translation software (www.deeple.com). Translations will subsequently be reviewed and validated and a translation validation form will be completed and signed by the reviewer. The IC procedure will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, confidentiality issues, etc. The ICF will include an option for the participant to consent with long-term storage of the samples for future research.
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 giving reasons and without any negative consequences. Participant Information Sheets (PIS) 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 study investigator or designee 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 (and no study-specific procedures will be performed) until the study investigator or designee has obtained his informed consent.
The ITM, or site-specific, Standard Operating Procedure (SOP) for 'Obtaining the Informed Consent of clinical trials' subjects will be followed.

A separate ICF will be available for the Focus Group Discussions (qualitative research part section 8.11).

8.3 Trial randomisation

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) No screening (intervention)
2) 3x3 screening (comparator)

The randomization list will be prepared using SAS 9.4 (SAS Institute, Cary NC). The randomization of the participants will be done within RedCap, a GCP-compliant electronic data capture software tool that the CTU has used to good effect in previous studies for this purpose. To ensure (approximate) treatment balance within study sites, the randomization list will be blocked by site using variable block sizes. The overview of the randomization list will not be shared with the investigators until the trial database is locked.

8.4 Blinding

This study will be unblinded.

Study Participants: The participant will not be explicitly informed to which intervention arm they have been randomized. However it will not be possible to completely blind the participants to which arm they are in, since those in the screening arm who test positive will be recalled for treatment of an asymptomatic infection. In addition, participants will be able to see which arm they are in the 3 weeks after enrolment if they login online to obtain their Ng/Ct results (a service that is in the process of becoming available to all PrEP recipients). If they are in the screening arm they will be able to see either a positive or a negative result for Ng/Ct. If they are in the no-screening arm, no test result will be visible. From this they will be able to deduce which arm they are in.

Doctors and study nurses: The attending doctors will not be blinded. Blinding the attending doctors would be very difficult within this study design as a week after each study visit, the doctors will receive the results of the Ng/Ct screening tests, but only for the participants in the 3x3 screening arm. At this point the doctor will be able to deduce which arm the participant is in. As a result, we have decided that the attending doctors will not be blinded to study arm allocation.

Statistician: The study statistician will be blinded until the statistical analysis plan (SAP) is approved.

8.5 Unblinding

Unblinding will not be necessary. If a participant urgently requires knowing if they are infected with Ng/Ct or not this will be done by repeat testing for these infections. If this occurs, this participant will not be excluded from the study.

8.6 Baseline data

The following baseline data will be collected during the screening/enrolment visit:

- Demographics: year of birth, gender,
- Sexual behaviour: number of sex partners in preceding 3 months, number of sex partners with condomless anal sex
- STI history: all STIs (Ct, Ng, syphilis, hepatitis C) diagnosed in the last 12 months before screening
- Antibiotics usage: all antibiotics used in the last 6 months before screening

8.7 Trial assessments

These assessments will be performed according to standard of care at each participating centre. In certain centres such as HSP the laboratory tests are performed the week before the clinic visit. These laboratory assessments will be considered to be the laboratory results for that particular visit. Participants in the non-screening arm will not be informed of their results at month 3, 6 and 9. They will only be informed about the result of the sample taken at month 0 and month 12.

Screening/enrolment visit (Day 0):

- Eligibility check
- Informed Consent Procedure
- Demographic and question regarding sexual behaviour
- PrEP questionnaire (only at ITM)
- Ask if symptoms compatible with STI
- Physical examination if symptomatic
- STI history last 12 months
- Antibiotic usage last 6 months
- Randomization
- Collection of results of syphilis and HIV testing (performed according to routine practice)
- Sample collection (according to standard practice of the participating sites.):
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)

Visit month 3 (can be between one week earlier and 6 weeks later):

- Ask if symptoms compatible with STI
- Physical examination if symptomatic
- STIs diagnosed since last visit (at study site or outside)
- Antibiotic usage
- Question regarding sexual behaviour
- PrEP questionnaire (only at ITM)
- Collection of results of syphilis and HIV testing (performed according to routine practice)

- Sample collection:
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)

Visit month 6 (can be between one week early and 6 weeks late):

- Ask if symptoms compatible with STI
- Physical examination if symptomatic
- STIs diagnosed since last visit (at study site or outside)
- Antibiotic usage
- Question regarding sexual behaviour
- PrEP questionnaire (only at ITM)
- Collection of results of syphilis and HIV testing (performed according to routine practice)
- Sample collection:
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)

Visit month 9 (can be between one week early and 6 weeks late):

- Ask if symptoms compatible with STI
- Physical examination if symptomatic
- STIs diagnosed since last visit (at study site or outside)
- Antibiotic usage
- Question regarding sexual behaviour
- PrEP questionnaire (only at ITM)
- Collection of results of syphilis and HIV testing (performed according to routine practice)
- Sample collection:
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)

Visit month 12 (can be between one week early and 6 weeks late):

- Ask if symptoms compatible with STI
- Physical examination if symptomatic
- STIs diagnosed since last visit (at study site or outside)
- Antibiotic usage
- Collection of results of syphilis and HIV testing (performed according to routine practice)
- Sample collection:
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)
- Question regarding sexual behaviour
- PrEP questionnaire (only at ITM)
- Experience of study participation and preference for screening vs. no screening of Ng/Ct (only at ITM)

Unscheduled visits:

- **Visits for treatment of detected STIs:**

  If any asymptomatic (in screening arm) or symptomatic (in both arms) 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. If the week-prior testing schema is used then the participant will be typically treated on the day of the clinic visit. These STIs will be treated according to our current guidelines [3]. Participants in the non-screening arm who come for STI testing at unscheduled study visits for example if they have symptoms of an STI, will be informed of the STI diagnosis according to local best practice guidelines.

  Persons with an HIV diagnosis will be offered optimal antiretroviral therapy and further best practice management according to our existing PrEP protocol [5]. They will exit this study after the diagnosis of HIV is made.

  As is currently the case for all PrEP recipients, participants of this study will be able to attend the PrEP/STI clinic at any point in between the scheduled visits for any health including mental-health related concerns. Participants will be encouraged to attend the clinic for any symptoms compatible with an STI.
  Any STIs (Ng, Ct, syphilis, HIV) diagnosed at non-scheduled visits between 0 and 12 months will be included in the primary and secondary outcome. Every effort will be made to obtain laboratory confirmation of these infections and only laboratory confirmed cases will be included in the study outcomes.

  Visits as a result of partner notification:
  Participants in the non-screening arm will also be tested and eventually treated empirically for Ng/Ct and other STIs if they are alerted for this purpose by partner notification. These visits will be classified as unscheduled visits and they will be tested for Ng/Ct at all three sites. They will also be encouraged to receive empiric therapy for Ng, CT or both according to which STI they were in contact with and what the nature of this contact was in accordance with IUSTI guidelines (https://www.iusti.org/regions/europe/euroguidelines.htm).

  Visits to assess test-of-cure:
  The Belgian National PrEP guidelines do not make a recommendation as to whether or not a test-of-cure is required following treatment for Ng or Ct. In practice most centres reserve performing a test of cure for gonorrhoea infections with a high risk of antimicrobial resistance (such as those from individuals epidemiologically linked to resistant infections) as well as infections with a documented elevated of borderline gonococcal MIC for ceftriaxone. In large part this limitation on performing routine tests of cure is related to patient preference. PrEP patients are followed up 3 monthly which is onerous on patients. They then need to come back for therapy every time they are diagnosed with Ng/Ct. If they need to add an extra test of cure visit to this schedule then many patients regard this as unacceptable or simply not achievable. We will continue with this approach and document all test of cure visits as unscheduled visits. When done, test of cure visits will be performed at the earliest 14 days after the receipt of effective Ng/Ct therapy to rule out

persisting dead DNA causing false positive NAAT test results. Because any positive Ng/Ct test from the test of cure visits is considered a non-resolved old infection, these will not be included in the primary or secondary outcomes as new infections. If however the test of cure was to assess if an Ng infection had cleared and a new Ct infection was detected at the test of cure visit then this would be included as a new infection in the primary and secondary study outcomes.

If a patient has evidence of clinical treatment failure this is a separate issue and we instruct patients to return for further tests if they have persistence of their symptoms. This is also not considered a new infection and will thus not be included in the primary or secondary outcomes as new infections.

All the following investigations are optional for any unscheduled visit:

- Ask if symptoms compatible with STI
- Physical examination
- STIs diagnosed since last visit (at study site or outside)
- Antibiotic usage
- Collection of results of syphilis and HIV testing (performed according to routine practice)
- Sample collection:
    - Urine sample (first-void) (Ng/Ct NAAT)
    - Pharyngeal swab (Ng/Ct NAAT)
    - Anorectal swab (Ng/Ct NAAT)
    - Pharyngeal or anorectal swab for every site that was Ng NAAT positive (Ng culture)

As is the case for routine PrEP visits currently, at every visit (scheduled or unscheduled) there will be some time for STI counselling and discussions of any concerns of the participants related to the study or not.

Assessing and confirming if a study participant has had a Ct/Ng infection in between study visits.

At the month 3, 6, 9 and 12 visits, the participants will be asked if they have had any symptoms of an STI since their last visit or if they have been tested for an STI since their last visit. If the answer to either of these questions is ‘yes’, then the study team will proceed with the Ng/Ct diagnosis verification protocol.

1. Obtain further information as to what the symptoms were, if any, and what anatomical sites were tested
2. Find out at which health provider (e.g. general practitioner (GP)) was the diagnosis made
3. Establish what antimicrobial therapy was received
4. Laboratory confirmation will be obtained of test results. The following will be regarded as laboratory proof of a Ct/Ng diagnosis:
    a. The participant has documented proof of a diagnosis of Ng/Ct based on a laboratory report.
    b. Laboratory evidence of a positive Ng/Ct diagnosis based on NAAT or Ng culture that can be obtained by the study team via: (1) online laboratory reporting systems

such as COZO, (2) telephonic contact with the laboratory or (3) telephonic contact with the health provider who performed the test.

8.8 Ng and Ct therapy in the study

The study will follow current best practice guidelines for the treatment of ***asymptomatic*** Ng and Ct infections:

Chlamydia
Doxycycline is recommended as first line therapy for chlamydia urethritis and proctitis [42, 43]. As such the study will recommend (in the absence of other clinical or other reasons) to treat these infections with 7 days of doxycycline 200mg per day per os. Wherever possible all positive chlamydia samples will be sent for assessment as to whether or not they are L-serovars.

Gonorrhoea
Wherever possible the current recommended therapy of ceftriaxone 500mg or 1g IMI x one dose plus azithromycin 2g PO x one dose will be used for Ng infections [44, 45].

For ***symptomatic*** Ng infections the same protocol will be used. For ***symptomatic*** chlamydia proctitis (such as a positive Ct NAAT), 7 days doxycycline may not be long enough to treat symptomatic LGV disease. As a result, the study doctors will either confirm that the Ct is not an LGV serovar (via NAAT) within 7 days and then treat with 7 days doxycycline or else treat with doxycycline 200mg per day for 21 days [46].

8.9 Table of trial procedures

Table 2. Trial procedures

Procedures	*Screening / Enrolment (V1)*	*Visit Month 3*	*Visit Month 6*	*Visit Month 9*	*Visit Month 12*	*Unscheduled visits (USV)*
Eligibility	x					
Informed Consent	x					
Randomization	x					
Physical examination (if indicated)	x	x	x	x	x	x
STI history	x	x	x	x	x	x
Antibiotic usage	x	x	x	x	x	x
Pharyngeal swab Molecular Ng/Ct Testing	x[1]	x	x	x	x	x
Anorectal swab Molecular Ng/Ct Testing	x[1]	x	x	x	x	x
Urine Molecular Ng/Ct Testing	x[1]	x	x	x	x	x
Results of syphilis and HIV testing (performed according to routine practice)	x	x	x	x	x	x
Question regarding sexual behavior: How many sexual partners since last visit)	x	x	x	x	x	
PrEP questionnaire at ITM	x	x	x	x	x	
FGD on experience and preference of screening at ITM					x	

1. Sample collected according to standard practice of the participating sites.

8.10 Laboratory procedures

Ng/Ct testing:

Laboratory testing for Ng/Ct from HSP and EH will be performed at the LHUB and for all specimens from the ITM, CHU, UZG at their STI laboratories.

- CT/NG molecular testing will be performed on urine, anorectal and pharyngeal samples.
- Each laboratory will pool each individual's pharyngeal, rectal and urine specimens at each visit and thereby conduct one NAAT (Nucleic Acid Amplification Test), instead of three, according to a validated pooling strategy. Two laboratories participating in this study have published studies validating this pooling strategy [47, 48]. Another pooling strategy validated by ITM will be published soon. Pooling will considerably reduce laboratory costs.
- In case a sample is found to be positive for Ct, the samples will be sent to the Reference STI Laboratory (ITM) where an additional test will be performed to differentiate L versus non-L strains according to current routine care at each centre.

- All study samples found to be positive for Ng or Ct will be stored at each centre until the end of the study and will then be sent to the ITM for long-term storage (future STI research).

HIV and syphilis testing:

- Syphilis, HIV testing will be performed according to each laboratory algorithm as in routine practice

8.11 Qualitative Research (only for ITM subjects)

Perceptions towards STI screening

At the ITM site, we will conduct a qualitative sub-study to address secondary objective 4, i.e. to explore PrEP users' perceptions towards STI screening. This is mainly to understand the feasibility of implementing a no-screening for Ng/Ct in PrEP cohorts.

We consider it highly unlikely that all MSM, and in this case PrEP users, will have an unanimous view on the utility of screening for Ng/Ct. The main focus is to explore the variety of perceptions towards STI screening, and how this may translate into preferences for being screened or not, rather than determining the extent to which participants agree with being screened for STIs.

Focus group discussions

Focus group discussions (FGDs) involve a meaningful process of 'sharing and comparing', encouraging participants to provide and explain their point of view. Hence, this method is most relevant for research objective 4. If needed, additional interviews may be conducted to explore more sensitive topics. An interdisciplinary qualitative research team will be set up, involving at least one sociologist (TR), one psychologist (CN), and the coordinating investigator (CK).

Informed Consent

An informed Consent Form for these FGDs will be used for every participant to these FGDs. The purpose of the FGDs will be described in the information sheet. In case of online FGDs, the information sheet will be forwarded to all potential participants. Potential participants will have sufficient time and opportunity to ask questions before the start of the FGDs. We will obtain verbal informed consent before the start of the online FGDs, or visually by asking participants to indicate that they consent by waving. We will document the consents provided in the consent form "Documentation of consent by the researcher".

Data collection

We will conduct three FGDs in the coordinating centre (ITM). We, therefore, assume that the diversity in perspectives towards screening for STIs does not differ substantially between the participating centres, although it cannot be excluded either. More FGDs may be conducted if deemed necessary by the qualitative research team. The first (FGD 1) will be conducted with PrEP users who declined participation in the Gonoscreen study (see below); the second (FGD 2)

with Gonoscreen participants; and the third (FGD 3) will be mixed with both (about 50% each). FGDs will be conducted in Dutch.

PrEP users who declined participation in the Gonoscreen study will be asked whether they can be invited to participate in this qualitative research part. Potential FGD participants will be explained that their point of view is of interest to the researchers and in no way wrong. They are free to decline at any time. The persons responsible for the informed consent procedure of the Gonoscreen study will keep a list with the contact details of those agreeing to be invited for this qualitative research part. This includes participants refusing participation in the Gonoscreen study. This list will be stored on a secured ITM server and password-protected. Participants who do agree to participate in the Gonoscreen study will be able to already indicate on the informed consent whether they can be invited to participate in the qualitative research part. Participants will be randomly recruited from both lists (e.g. every tenth PrEP user).

The FGDs will be conducted in a private environment (eg. a meeting room at the study site). The topic guide will include questions to enhance discussion on sexual health, STI prevention and the role of screening for STIs. Given the sensitivity of the topic we will use group exercises and cases, in which people can refer to third-persons rather than personal experiences. The topic guide will be pilot tested during one FGD with at least 6 PrEP users or research team members. The moderators will be researchers with considerable experience in qualitative research and knowledge of the subject. They will carefully facilitate the group dynamics in a way that participants can freely express themselves and their potentially different opinions on screening strategies.

To mitigate the difficulties of conducting the FGDs in the COVID-19 period, we foresee the possibility to conduct these FGDs via an online platform ‘ZOOM’, which is HIPAA (Health Insurance Portability and Accountability Act) compliant. This will be a professional ZOOM package, using restricted settings for higher security (e.g. waiting room function, meeting ID and required password for joining a session). We will arrange the settings such so that participants can see each other (if they consented) and the interviewer(s) to encourage the dynamic of the group discussion. We will also encourage participants to use a background function in order to not reveal details of their private homes to increase their privacy.

Data analysis

Each focus group discussion will be transcribed and coded before conducting the next, allowing for a constant comparative approach [49]. Such iterative process of data collection and analysis enhances the validity of the findings. After each round, the research team will be asked to review the analyses and to ensure that the findings are relevant (face validity). The analysis will be focused on finding differences in the perceived importance of screening for STIs and how this may be translated into screening strategies.

Data management and storage

The form “documentation of consent by the researcher” will be stored along with the informed consents of the main study. The audio and video files of the FGDs will be stored securely on a personal server of ITM of the coordinating social scientist (TR), password-protected. During transcription, it will be verified that there is no information that can directly identify any participant.

The pseudonymised transcripts will be stored on the interdepartmental ITM server, in a folder of the study, to which only the research team has access. After the transcription has been verified by the coordinating social scientist (TR), the audio and video files will be deleted. The transcripts will be stored for 5 years after completion of the study, as a back up.

8.12 Withdrawal criteria

8.12.1 Discontinuation of trial procedures

The Investigator has the right to withdraw participants from the study if the following event occur:

- The participant tests HIV positive before the 12th month visit (see below)
- The Investigator judges that further participation would have negative effect on the participant's mental and/or physical health
- The participant wishes to stop the trial procedures

In the event of a study participant discontinuing the trial procedures, their data will be used in the analysis up to the timepoint they withdraw.

8.12.2 Withdrawal of consent (discontinuation of trial participation)

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. For the purposes of this trial, withdrawal is defined as: The participant would like to withdraw consent from study and is not willing to be followed up for the purposes of the trial at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the trial analysis).

The details of withdrawal should be clearly documented in the participant's medical records and in the electronic Case Report Form (eCRF).

Handling of Withdrawals

A complete final evaluation should be made at the time of the participant's withdrawal (discontinuation of all study procedures). The End of Study form in the case report form should be completed with an explanation of why the participant 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.

8.12.3 Loss to follow-up

A participant 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.

8.12.4 Termination of the 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 trial steering committee after consultation with the independent safety experts (DSMB) (See Appendix 1).

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions and the EC's of the termination or suspension and the reason(s) for the termination or suspension.

8.13 End of trial

The end of the trial is considered the date of the last visit of the last participant. The applicable ECs/ Institutional Review Boards (IRBs) will be informed within 90 days of the end of trial.

9 TRIAL INTERVENTION

9.1 Name and description of intervention(s)

The intervention in this trial will be to ***not*** screen PrEP recipients for STIs (Ng and Ct) every 3 months compared to the current Belgian guidelines of screening every three months at 3 sites (3x3 screening).

9.2 Legal status of the intervention

Belgian HIV Pre Exposure Prophylaxis (PrEP) guidelines currently recommend 3x3 screening (screening 3-sites -pharynx, rectal and urethral- at 3 monthly intervals) for all PrEP recipients. The intervention is thus not in line with the current Belgian guidelines. The study was however developed within BREACH (who were responsible for the development of the Belgian Guidelines) as a means to provide empirical evidence as to whether or not the currently recommended 3x3 screening strategy is associated with a reduction in incidence of Ng plus Ct.

9.3 Intervention schedule

Participants in the intervention arm will ***not*** be screened for Ng/Ct for a period of 12 months, in reference to the routine practice of screening (and treating screening-positive) participants every 3 months.

More specifically, participants in the intervention arm will have 3 site (urethra, pharynx and rectum) sampling performed every 3 months but these testing results will only be reported at the end of the 12 month period.

10 SAFETY RECORDING AND REPORTING

10.1 Recording of safety findings in function of the available evidence

10.1.1 General considerations for the recording of safety findings

The largest safety concerns for this study are that the participants in the non-screening arm (and their partners) may experience an increase in the incidence of symptomatic Ng/Ct. Since

symptomatic Ng/Ct infections occur fairly frequently (over 10% symptomatic urethral Ng per year [50]) even in the presence of 3x3 screening in MSM PrEP cohorts, it is not optimal to define them as adverse events of non-screening.

Rather than reporting each symptomatic episode of Ng/Ct as an adverse event, the independent DSMB will evaluate if the non-screening arm has an unacceptably high incidence of symptomatic Ng/Ct. For this purpose, the DSMB will include two independent STI experts (Infectious Disease Physicians/Epidemiologists) and the study statistician who will together decide what threshold they determine to be an unacceptable risk and what methodology to use to evaluate this risk. As the number of STIs occurring during the study is also the main outcome of the trial, all STIs will be recorded both in the source documents and in the eCRF.

Our proposal will be to evaluate the incidence of symptomatic Ng + Ct in both arms at two time points: once 50% and 100% of all study recruits have passed their 6 month visit. If the incidence of symptomatic Ng + Ct in the non-screening arm is more than two-fold higher than the screening arm then serious consideration should be given to stopping the study.

10.2 Responsibilities

Principal Investigator (PI):

The PIs and delegated study doctors at each study site will be responsible for ensuring that all episodes of symptomatic Ng/Ct infections are adequately investigated, treated, followed up and reported so as to allow a timeous assessment of any excess incidence of symptomatic Ng/Ct in the non-screening arm.

They will also be responsible for correct documentation of these STIs in the source documents and in the corresponding eCRFs.

Sponsor:

1. The sponsor will be responsible for the central data collection of these STIs through the eCRF database.
2. The sponsor will be responsible for informing the Trial Steering Committee (in particular the two independent experts) at 6 and 9 months of the amount of STIs that have occurred since the start of the study.

TSC:

The TSC (two independent STI experts in particular) will be responsible for evaluating the STI incidence at regular intervals and advising the sponsor on continuation of the trial.

10.3 Notification of deaths

Any deaths of study participants will be reported within 7 days to the sponsor. In case a participant dies during the study, this will be reported in the source documents and in the eCRF.

10.4 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant EC of the measures taken and the circumstances giving rise to those measures.

11 STATISTICS AND DATA ANALYSIS

Statistical analysis of the study will be performed by the biostatistician at the CTU according to a SAP approved before unblinding of the study statistician.

11.1 Sample size calculation

The primary endpoint is the number of diagnoses of Ng plus Ct over the study period (12 months) whilst individuals are screened vs. not screened.

> ***Numerators:*** Cumulative number of diagnoses of Ng plus Ct in 12 months in screening/non-screening arms. Each participant can only contribute one diagnosis of Ct and one diagnosis of Ng per (scheduled or unscheduled) visit - regardless of number of sites infected. Thus each participant can contribute up to 2 diagnoses (Ct/Ng) at each (scheduled or unscheduled) visit. The numerator includes laboratory-confirmed diagnoses made between scheduled visits, performed inside or outside of the study.
>
> ***Denominators:*** Number of scheduled study visits with available results for the diagnosis of Ng/Ct. The denominator does not include unscheduled visits.

Testing for these infections is performed at each three-monthly visit, i.e. 4 follow-up visits per subject (if complete follow up). Ng/Ct are tested for at 3 sites at each visit – rectum/pharynx and urethra. If a participant is found to be infected with one of these infections at more than one site on a particular visit this is counted as a single infection. If they are found to have both Ng and Ct at a visit this is classified as 2 infections.

Based on the Be-PrEP-ared study from ITM, we assume at baseline visit 79% have 0 diagnoses (pre treatment), 18% have 1 diagnosis (either NG or CT) and 3% has 2 diagnoses (both NG and CT). With screening, the number of diagnoses in the Follow Up (FU) visits depends on number of diagnosis at baseline and is shown below.

	0 diagnoses[#]	1 diagnosis[#]	2 diagnoses[#]	Average nr of diagnoses
0 diagnoses at baseline	86%	13%	1%	0.15
1 diagnosis at baseline	76%	20%	4%	0.28
2 diagnoses at baseline	71%	24%	5%	0.34

[#] *These columns refer to the percentage of participants who have 0/1/2 diagnoses at each follow-up visit. In other words, of those with 0 diagnoses at baseline (first row), at each of the visits at 3,6,9 and 12 months, 86% will have 0 diagnoses, 13% will have 1 diagnosis and 1% will have 2 diagnoses.*

Over 4 FU visits, this yields an average of 0.72 diagnoses per subject. Using a Poisson regression model with log(number of visits) as offset, the 95% confidence interval of the log ratio (no screening vs. screening) is calculated. The 'no screening' arm is considered to be non-inferior if there is an increase of maximal 25% in number of diagnoses (i.e. increase of an average of 0.72 to 0.90 per 4 visits). The 'no screening' arm is proven to be non-inferior if the upper limit of the 95% confidence interval is lower than log(1.25). The required sample size to detect non-inferiority of the 'no screening' arm is calculated by simulation, assuming no difference between arms.

Assuming that 95% of the participants will have data on all 4 visits, and 5% will have data on only 3 visits, the required sample size to obtain 80% power is 912. Assuming an additional 10% drop out rate we will need to enroll 1014 participants.
The current number of PrEP participants at the ITM/HSP/EH/CHU/UZG is 803/739/290/260/240, respectively. At all 5 centres this number is increasing by approximately 20% per year. 99.7% of these individuals are MSM and over the age of 18. We estimate that 80% of the 2332 PrEP participants in these five centres will agree to participate. This number (n=1866) exceeds the number we need to enroll in the study (n=1014). By the commencement of the study (begin 2020) we estimate we will have 1518/886/464/314/298 individuals in the PrEP cohorts at the ITM/HSP/EH/CHU/UZG whom we will be able to screen for participation.

11.2 Planned recruitment rate

The 5 centres will begin recruitment at the same time and will continue recruiting all PrEP participants until the recruitment target is reached (estimated 8-month recruitment period). The planned recruitment rate will be 127 individuals per month for this 8-month period. To the best of our knowledge, there are no other trials being conducted currently or planned that are evaluating the same research question or a version thereof.

11.3 Statistical analysis plan

11.3.1 Summary of baseline data and flow of participants

Participants in each intervention group, and in each site, will be described with respect to baseline characteristics. The description will be in terms of medians/means and quartiles/standard deviations for continuous characteristics and using counts and percentages for categorical characteristics. The clinical importance of any imbalance will be noted though statistical tests of significance will not be undertaken.

Details of participants screened, those who meet the study inclusion criteria, those who are eligible and randomized, those who are eligible but not randomized, those who withdraw from the study after randomization and those who are lost to follow-up will be summarized in a CONSORT flow diagram. The number (%) of participants attending scheduled follow-up visits will be reported.

11.3.2 Primary outcome analysis

We will analyse the primary outcome both using Intention-to-Treat and Per-Protocol approaches, with Per-Protocol as primary approach. In the Intention-to-Treat analysis, all participants will be analysed according to their randomized allocation, even in case they receive another intervention, show protocol violations prior to or during the study, or are lost-to follow. In the per-protocol analysis only participants who receive intervention as planned, and follow the protocol as planned

are included. If the person urgently requires knowing if they are infected with Ng/Ct or not this will be done by repeat testing for these infections. If this test is positive for Ng/Ct then this will be counted as an intervisit infection in the final analysis. In each arm, the incidence of Ng plus Ct will be estimated together with 95% confidence interval. Estimates will be based on a Poisson regression model with number of diagnoses as dependent variable, arm as independent variable, log(number of visits) as offset and a random intercept for site. This model will also provide an estimate of the log incidence rate ratio (no screening versus screening), together with 95% confidence interval. The 'no screening' arm is proven to be non-inferior if the upper limit of the 95% confidence interval is lower than log(1.25).

Visits with missing data on the diagnosis of Ng or Ct, will be excluded from the calculation of the incidence rate (excluded both from numerator and denominator).

Subgroup analysis:

We will conduct a prespecified sensitivity analysis to assess the incidence of Ng plus Ct in screening vs. non-screening arms ***in participants with lower-risk behavior***. An individual with lower-risk behavior is defined a priori as those individuals who report 4 or fewer partners per 3-month period in all 5 relevant 3 month periods. These periods are the 3-months prior to study enrollment as well as the four 3 month periods during study follow up). The analysis will be analogous to the analysis of the primary objective, but restricted to participants with lower-risk behavior.

11.3.3 Secondary outcome analysis

1) We will compare the antimicrobial exposure (ceftriaxone/azithromycin/doxycycline) in the two arms. Antimicrobial exposure will be measured as number of standard doses per 1000 person-years based on standard WHO and ECDC methodology [41]. Data on all antimicrobials consumed will be collected at each study visit and will include antimicrobials consumed at other health facilities such as at participant's General Practitioner. A Poisson regression model with number of standard doses as dependent variable, arm as independent variable, log(time at risk) as offset and a random intercept for site will be fitted.
2) Incidence rate of symptomatic Ng and Ct. This information will be collected at all visits and will include episodes of symptomatic Ng and Ct diagnosed elsewhere as long as these were laboratory confirmed diagnoses – either based on molecular testing or in the case of Ng, culture. The analysis will be analogous to the analysis of the primary objective, but the endpoint is calculated using symptomatic infections only.
3) Incidence rate of syphilis. The European IUSTI case definition of a syphilis infection will be followed [51]. A Poisson regression model with number of incident syphilis infections as dependent variable, arm as independent variable, log(time at risk) as offset and a random intercept for site will be fitted.
4) Incidence rate of HIV. A Poisson regression model with incident HIV infection as dependent variable, arm as independent variable, log(time at risk) as offset and a random intercept for site will be fitted.
5) Economic impact of cessation of 3x3 screening in MSM in Belgium (see section 11.4)
6) Participants' perception/experience and preference for screening vs. no screening (see section 8.11, only for ITM subjects)

11.3.4 Procedure(s) to account for missing or spurious data

PrEP recipients are required to attend follow up 3 monthly and adherence rates with this requirement are high. This is in part related to the fact that they need to attend 3-monthly to receive their prescriptions for the next 3 months. In the study we will allow study visits to be from one week before to 6 weeks after this 3-month duration. This will decrease the proportion with missed visits.

Study participants who miss their follow up visits (defined as more than 6 weeks after their 3 month follow up visit) will be contacted to attend as soon as possible. If they miss a visit, the reason for this will be recorded in the source document and eCRF.

Missed visits will be excluded from the denominator of incidence.

11.3.5 Other statistical considerations

Any deviations from the statistical analysis plan will be justified in the statistical analysis report.

11.4 Data collection for economic evaluation

One of the goals of the KCE Trials programme is to improve the efficiency of the healthcare system. This protocol has been designed with a later possible economic analysis in mind. The planned economic analysis is briefly described below, together with the variables collected in this protocol for this purpose. For the sake of clarity, the economic analysis is not a part of this trial. The decision to conduct such economic analysis will depend on the effectiveness results of this trial.

The major expected costs and benefits of 3x3 screening in MSM are detailed in Table 3. Given the available evidence, and in particular the lack of any RCTs assessing the effectiveness of screening, we are unable to a priori know if screening does result in the first two benefits listed in the table – reduction of spread of Ng/Ct and fewer episodes of symptomatic disease. As outlined in the 'Background' section, screening may result in arrested immunity and thereby paradoxically increase the incidence of symptomatic infections and the spread of Ng/Ct. The current RCT and its envisioned second phase, will thus provide useful empirical estimates of what these effect sizes and directions are. The direct costs of screening include the cost of testing, consultation fees for the initial visit, the visit to receive treatment (for those testing positive) and in the case of a positive Ng molecular test perform culture and sensitivity, the follow up visit 2 weeks later to assess treatment response (for Ng infections), the price of antimicrobial therapy – including cost of administering injections (for Ng). The indirect costs of screening include the time taken to attend consultations and the adverse effects of the high levels of antimicrobials on the individual's health, including their microbiome/resistomes and the selection for antimicrobial resistance in Ng/Ct, other STIs and other bacteria in their microbiomes. This adverse effect will operate at both an individual and population level.

The important link between perception of Ng/Ct screening efficacy and impact on QoL

Screening in and of itself would not be predicted to have a direct effect on a PrEP participant's quality of life. If, however, a participant knew or thought that screening reduced his risk of symptomatic Ng/Ct or of Ng/Ct transmission to his partners then participating in screening could improve his quality of life via the following two plausible cognitions:

- “By screening I am protecting my partner”
- “By screening I am protecting myself from symptomatic Ng/Ct”

As already noted above, we do not know if screening has these beneficial effects. In our discussions with PrEP recipients it has emerged that many believe that we screen for Ng/Ct based on good evidence of efficacy in terms of these two parameters. They typically express considerable surprise when we explain this is not the case. This creates a significant problem for health economic evaluations that compare quality of life in screening vs. non screening arms. If participants falsely believe that screening is highly efficacious then it is possible that a quality of life (QoL) assessment in our study would reveal a higher QoL in those screened vs non-screened. This could occur despite the study finding no difference in symptomatic Ng/Ct and onward transmissions (as assessed in phase 2).

If the study was then repeated with the same individuals but with this new information given to them (that screening does not reduce transmission or symptomatic disease) then those screened may now report a lower QoL than those in the non-screening arm. This could be explained by this individual no longer having the two cognitions and instead having the cognitions:

- “Screening will not reduce my partners probability of acquiring Ng/Ct”
- “Screening will not reduce my probability of getting symptomatic Ng/Ct”

In this new scenario, QoL may be further reduced in those screened due to the money and time spent on screening as well as side effects from and perceptions related to the adverse effects of antibiotics.

These considerations lead us to conclude that the key information we require at this stage is the efficacy of Ng/Ct screening and that including QoL assessments in our study design would not be appropriate.

In the event of us finding non-inferiority in our primary outcome, we will conduct an analysis to assess what the cost savings of the cessation of screening for Ng/Ct in MSM in Belgium would be.

If we find that no-screening is non-inferior to 3x3 screening, we will be able to recommend the cessation of Ng/Ct screening in the approximately 1526 individuals in PrEP follow up in Belgium and save these direct costs. A reasonable case could also be made based on these findings that Ng/Ct screening could also be stopped in MSM more generally. Since local and European guidelines recommend 3 site screening for all sexually active MSM from 3 to 12 monthly and there are an estimated 148 081 MSM in Belgium this could result in considerable cost saving to the health care system [52]. Furthermore, the number of individuals on PrEP is expected to continue to grow to cover approximately 20% of the MSM population [53].

We also plan to model the effects of stopping screening on the selection of antimicrobial resistance in Belgium. The major motivation and the major anticipated utility of the trial is the reduction of antibiotic use in a key population where antimicrobial resistance has frequently emerged [54]. By reducing antibiotic usage, we aim to reduce the probability of the emergence of antimicrobial resistance in Ng as well as a range of other STIs and other infections. Whilst it is difficult to attach a cost saving value to an intervention that prevents the emergence of highly resistant and untreatable infections such as Ng, it is important to do so. The O'Neill Report, for example, predicted that infections due to antimicrobial resistant bacteria will result worldwide in 300 million deaths and a cumulative cost of $100.2 trillion by 2050 if we continue on the current

trajectory [55]. We will attempt to quantify these long-term effects for the Belgian population by adapting our model of Ng transmission in MSM in Belgium [56] to model the probability of emergence of resistance given various intensities of antimicrobial consumption/screening intensity.

If we find that screening is associated with a reduction in the incidence of Ng plus Ct, we will conduct cost-effectiveness evaluations such as calculating the cost per averted infection.

We plan to collect the costs relating to laboratory testing, price of antimicrobial therapy and administration and costs of consultations from standard sources such as RIZIV/INAMI reimbursement schema and BCFI.

Table 3: Major costs and benefits of 3x3 screening in MSM

Costs	Benefits
Direct	Possible reduced risk of symptomatic Ng/Ct (individual level benefit)
Cost of 3 site screening (molecular testing)	Possible reduced transmission of Ng/Ct (population level benefit)
Cost of culture & sensitivity testing for positive Ng molecular tests	Screening results in individuals with higher risk behaviour attending follow up regularly
Consultation fee initial screening visit	
Consultation fee visit to receive treatment	
Follow up visit to assess test of cure (Ng)	
Price of antimicrobial therapy	
Indirect	
Time to attend consultations	
Adverse effects of high level of antimicrobial consumption on individual's health including microbiome	
Adverse effects of high level of antimicrobial consumption on selection of antimicrobial resistance (individual and population level cost)	
Certificate of health effect may result in increased risk behaviour	

12 DATA HANDLING

12.1 Source document identification

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.

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, STI history, antibiotic usage,

randomization and lab test results), Follow-Up visit data (physical examination (if clinical indication), STIs diagnosed since last visit, antibiotic usage, lab test results and Outcome data (final outcome).

12.2 Data handling and record keeping

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

- Responsible persons

  Data Management will be performed by the trial staff at the site (data entry), in collaboration with a data manager from the CTU at the ITM (data review and cleaning).

- 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 (i.e. restricted access). A list of authorized users and their user roles will be kept at the CTU and updated as needed.
  The encrypted trial data will be stored on a secured server at the ITM, which is only accessible to IT administrators. The IT department has procedures in place to ensure daily backup of the server data 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. No direct identifiers of the participant will be included on the eCRFs, 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

  Data management will be done in compliance with Good Clinical Practice (GCP) guidelines and the European General Data Protection Regulation 2016/679 (GDPR).

  A study specific data management 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.

  Metadata (e.g. variable name, variable description, label, data type (date, string, number), etc.) will be defined in a data dictionary and human-readable codebook.

12.3 Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution to permit trial-related monitoring, audits and inspections.

We recognize the importance, societal and moral obligation of making accessible its collected research data openly and transparently with the wide research community. Second to research outputs and findings, we are committed to share research data underlying research findings with as few restrictions as possible, and in a timely manner.

With regard to open access to data, we adhere to the European FAIR principles (**F**indable – **A**ccessible – **I**nteroperable and **R**eusable) and recognize that data should be *"As open as possible and as closed as necessary".*
As such, ITM's data sharing policy will be followed according which the data supporting the findings of this study will be retained at the Institute of Tropical Medicine, Antwerp and will not be made openly accessible due to ethical and privacy reasons. Data can however be shared by means of a controlled access procedure. Thus, participant level data can be made available after approval of a motivated and written request to the Data Access Committee of the Institute of Tropical Medicine at ITMresearchdataaccess@itg.be and after the conclusion of a Data Sharing Agreement. Data will be anonymized as much as possible with regard to data sharing for secondary research.

12.4 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 (IF) 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 electronic 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 years.

13 MONITORING, AUDIT & INSPECTION

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 (SDV).

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 centre inspection. Likewise, the investigator will inform the sponsor of any pending inspection.

Laboratory quality control and quality assurance:
All participating laboratories 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 and testing will be conducted in compliance with Good Clinical Laboratory Practice Standards (GCLP), and EN-ISO 15189. All laboratory processes and analyses of the study will be conducted in compliance with the laboratory analytical plan. Reports of laboratory test results will be forwarded to the study physician as soon as the result is available.

14 ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Summary of risks and benefits of participation in study

A detailed assessment of the risks and benefits of study participation are provided in sections 2 and 3. In this section we provide a summary of these risks and benefits.

The ***main risks*** involved for participants is they may develop symptomatic gonorrhoea or chlamydia infections or transmit these to one of their partners. There is an argument that these could be prevented by screening. This has not however been established empirically. Likewise the incidence of these STIs could increase due to the cessation of screening.

The ***main benefits*** of participation are that those in the non-screening arm will likely be exposed to less antimicrobials than is currently the case. Less screening of infections that are mostly asymptomatic and self-resolving means that likely fewer of these infections will be diagnosed and treated. Antimicrobial consumption has been linked to a number of adverse clinical outcomes. Participants will also contribute to a study which, for the first time, will evaluate whether screening for gonorrhoea and chlamydia in this population is associated with a reduction in the incidence of these infections? This is a question which PrEP recipients ask frequently and it is thus a question which they would like seen answered by appropriate clinical trials such as the GonoScreen trial.

14.2 Ethics Committee (EC) review & reports

This clinical trial (trial protocol, informed consent forms and other relevant documents) will be submitted for formal review and approval to the Institutional Review Board of the ITM, the EC of

the University Hospital of Antwerp, the local ECs of Erasmus Hospital, St. Pierre Hospital, CHU Liège and UZGent. No study-specific interventions will take place before written approval by the Ethics Committees 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.

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.

An annual progress report (APR), prepared by the Coordinating Investigator, will be submitted to the relevant ECs within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

The end of the study (including in case the study was ended prematurely) will be notified to the IRB and EC by the Coordinating Investigator and a final study report with the results will be submitted to the EC within one year after the end of the study. The end of study will be defined as the last visit of the last participant.

All correspondence with the IRB and EC will be retained in the Trial Master File/Investigator File.

14.3 Peer review

The current protocol has been reviewed in a number of different forums.

1. The protocol has been reviewed by a panel of independent reviewers appointed by KCE. Our responses to the initial review process have been subsequently reviewed by this panel.
2. The protocol has been developed in conjunction with BREACH. Each of the 3 AIDS Reference Centres participating in the study has reviewed the proposal. In addition, the proposal was discussed with two other AIDS Reference Centres.
3. Ethical considerations pertaining to the study were reviewed by both the IRB of the ITM and the Ethics Committee of the UZA.
4. The final protocol was discussed with two international experts in the field of Chlamydia screening/STI epidemiology (one expert) and STI Physician (one expert)
5. The study protocol was reviewed by the Scientist responsible for STI Surveillance at SCIENSANO (Appendix 5)

14.4 Public and Participant Involvement

This RCT protocol has emerged as a result of discussions with a number of PrEP recipients who have asked for the evidence we have as to the efficacy of screening for Ng/Ct in MSM. We typically answer that we have no RCT based evidence to back up our current practice and explain that each time we screen this results in an approximately 20% chance that they will require antimicrobial therapy which will have an adverse effect on their resistome (collection of all AMR genes in bacteria) for up to 4 years [15]. This information has resulted in a number of PrEP recipients asking questions along the lines of: "How can you do this if you do not have evidence that this is beneficial to us?" This trial would provide an answer to this question. We have also presented the idea for this RCT at the 6th Belgian Research Aids & HIV Consortium (BREACH) Symposium. The idea of conducting a RCT in Belgian PrEP centres was supported at this meeting and the preparation and submission of the project was formally approved at the 2018 BREACH Annual General Meeting.

Two meetings have been held with SENSOA (Vlaams Centrum voor Seksuele Gezondheid) to discuss this research proposal and develop the RCT protocol. One of the meetings at SENSOA was with SENSOAs MSM Liaison Officer Marc Sergeant, who provided valuable input on study design.

The proposal has also been reviewed by SCIENSANO.

14.5 Regulatory Compliance

The trial will not commence until authorisation is obtained from the IRB and EC.

The protocol and trial conduct will comply with the Belgian law of May 7th 2004 regarding experiments on the human person and any relevant amendments. Since there is no Investigational Medicinal Product (IMP) involved, the trial does not fall under the Clinical Trials Regulation of the European Union and the latest Belgian law of May 2017. As such, the trial will not be submitted to the Belgian authorities (FAMHP).

14.6 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

- prospective, planned deviations or waivers to the protocol are not allowed and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol
- accidental protocol deviations can happen at any time. They must be adequately documented and explained on the relevant forms and reported to the Coordinating Investigator and Sponsor immediately.
- deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

14.7 Notification of Serious Breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree –

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial

In this case, the sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase. The sponsor will have serious breaches investigated by an independent body.

14.8 Data protection and participant confidentiality

The controller is the Institute of Tropical Medicine with regard to the processing of the personal data in the context of this research study. The lawful basis for the processing of participant data is the public interest. A collaboration agreement concluded between all the study partners and prior to subject screening will include a section on data processing in compliance with the GDPR.

Information of trial participants will be handled confidentially. A trial participant code will be assigned to each trial participant at the earliest opportunity (pseudonymization). 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.

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.

All investigators and trial site staff must comply with the requirements of the legislation on the protection of privacy in relation to the processing of personal data (GDPR and the Belgian act of 30-JUL-2018 on the processing of personal data; see also https://www.dataprotectionauthority.be/legislation-and-standards). The Data Protection Officer at the ITM is Jef Verellen (informatieveiligheid@itg.be or +32 (0) 3 247 07 43).

See also chapter 12 for technical and organisational measures with regard to data management and data sharing.

14.9 Financial and other competing interests for the Coordinating investigator, PIs at each site and committee members for the overall trial management

The Coordinating investigator and PIs at each site declare that they have no conflicts of interest. In particular we have no competing financial interests.

14.10 Insurance

The Sponsor of this study, the Institute of Tropical Medicine has obtained a (no-fault) study insurance to cover any injury, damage or loss to study participants and which is caused directly or indirectly by their participation in the study.

14.11 Access to the Study Data by KCE and similar institutes in the EU

This section should be read in conjunction with the research agreement, which supersedes the protocol in case of contradictory statements.

A distinction is to be made for access by KCE (and similar institutes in Europe) and access by other parties.

Access to Study Data by KCE is fully defined in the contract between KCE and the Sponsor and the research agreement template is publicly available on the KCE website. Link: https://kce.fgov.be/en/resources-for-investigators

14.12 Access to the final trial dataset by other parties

After study completion and publication of results, anonymized or pseudonymized 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. Access requests will be reviewed and approved prior to release by ITMs Data Access Committee. Requests for access can be made centrally through: https://www.itg.be/E/data-sharing-open-access .

15 DISSEMINATION POLICY

15.1 Dissemination policy

Following finalization of the end of study report, the study results will be disseminated as soon as possible. Particular efforts will be made to communicate the study results to those who participated in the design and conduct of the study. This will include SENSOA, SCIENSANO, RIZIV/INAMI, Cavaria, Boys Project and Roze Huis. Pamphlets with the key study findings will be prepared and made available to participants and others in all PrEP waiting areas in all participating centres.

The final study report will be made available for review and comment by KCE before dissemination if this is required by the agreement KCE. The TSC will develop a study publication plan which will adhere to the following principles:

- Publications follow the 'European Code of Conduct for Research Integrity'
- All publications will acknowledge KCE's funding of the study and as appropriate carry a KCE disclaimer.
- Open access journals will be preferred
- The Consort Guidelines will be followed: *http://www.consort-statement.org/*

The trial will also be registered in the public registry ClinicalTrials.gov prior to the start of enrolment.

15.2 Authorship eligibility guidelines and any intended use of professional writers

The TMG written Study Publication plan will be guided by the ITMs 'Guidelines regarding authorship in scientific publications' document. A key component of this document is that it adheres to the following criteria for authorship:

The basic criterion for authorship is a significant intellectual contribution to the work reported. This contribution has to include at least one of the following points:

- conceptualization of (part of) the work;
- design of the study;

- execution of the study;
- data analysis;
- data interpretation;
- a part in the writing of the article;
- critical revision of the article for content.

It is up to the authors to agree what contribution was significant. In case no consensus is reached then the senior researcher can have a decisive role in the decision.

As a general principle group authorship will be promoted and at least one individual from each participating centre should be an author on all papers.

16 REFERENCES

1. Fairley CK, Cornelisse VJ, Hocking JS, Chow EPF. Models of gonorrhoea transmission from the mouth and saliva. Lancet Infect Dis. 2019. Epub 2019/07/22. doi: 10.1016/S1473-3099(19)30304-4. PubMed PMID: 31324517.
2. Tsoumanis A, Hens N, Kenyon CR. Is screening for chlamydia and gonorrhea in men who have sex with men associated with reduction of the prevalence of these infections? a systematic review of observational studies. Sex Transm Dis. 2018;45(9):615-22.
3. ECDC. HIV and STI Prevention among Men Who Have Sex with Men. doi:102900/66666. 2015.
4. Kenyon C. How actively should we screen for chlamydia and gonorrhoea in MSM and other high-ST-prevalence populations as we enter the era of increasingly untreatable infections? A viewpoint. Journal of medical microbiology. 2018;68(2):132-5.
5. Belgian Research AIDS & HIV Consortium. Use of Pre Exposure Prophylaxis (PrEP) : Belgian guidelines. 2017.
6. Kenyon C. We need to consider collateral damage to resistomes when we decide how frequently to screen for chlamydia/gonorrhoea in PrEP cohorts. AIDS. 2019;33:155-7.
7. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC infectious diseases. 2014;14(1):13.
8. Kenyon C. Prevalence of macrolide resistance in Treponema pallidum is associated with macrolide consumption. Journal of medical microbiology. 2018;68(2):119-23.
9. Kenyon C, Buyze J, Spiteri G, Cole MJ, Unemo M. Antimicrobial consumption is associated with decreased antimicrobial susceptibility of Neisseria gonorrhoeae in 24 European countries: an ecological analysis. JID. 2019;Apr 8. pii: jiz153. doi: 10.1093/infdis/jiz153. [Epub ahead of print].
10. Kenyon C, Buyze J, Wi T. Antimicrobial consumption and susceptibility of Neisseria gonorrhoeae: a global ecological analysis. Frontiers in medicine. 2018;5:329.
11. Kenyon C. Association between antimicrobial consumption and AMR in gonorrhoea in Australian states F1000Research. 2019; 8:604 (poster) doi: 10.7490/f1000research.1116708.1.
12. Kenyon C, Laumen J, Van Dijck C. Could intensive screening for gonorrhoea/chlamydia in PrEP cohorts select for resistance? Historical lessons from a mass treatment campaign in Greenland. STDs. (In Submission).
13. Kenyon CR, De Baetselier I, Crucitti T. Does gonorrhoea screening intensity play a role in the early selection of antimicrobial resistance in men who have sex with men (MSM)? A comparative study of Belgium and the United Kingdom. F1000Research. 2018;7.
14. Kenyon CR. Association between intensity of STI screening and development of antimicrobial resistance in N. gonorrhoeae in 12 cities in the USA: An ecological study. F1000Res. 2018;7:1237. Epub 2019/01/11. doi: 10.12688/f1000research.15569.4. PubMed PMID: ; PubMed Central PMCID: PMC
15. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One. 2010;5(3):e9836. doi: 10.1371/journal.pone.0009836. PubMed PMID: 20352091; PubMed Central PMCID: PMC2844414.
16. Kenyon C. Toward a set of criteria to decide which STIs to screen for in PrEP cohorts Front in Public Health. 2019;7:154.
17. Unemo M. Current and future antimicrobial treatment of gonorrhoea - the rapidly evolving Neisseria gonorrhoeae continues to challenge. BMC Infect Dis. 2015;15:364. doi: 10.1186/s12879-015-1029-2. PubMed PMID: 26293005; PubMed Central PMCID: PMCPMC4546108.
18. Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. Clin Microbiol Rev. 2014;27(3):587-613. doi:

10.1128/CMR.00010-14. PubMed PMID: 24982323; PubMed Central PMCID: PMCPMC4135894.
19. Wi T, Lahra MM, Ndowa F, Bala M, Dillon JR, Ramon-Pardo P, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. PLoS Med. 2017;14(7):e1002344. Epub 2017/07/08. doi: 10.1371/journal.pmed.1002344. PubMed PMID: 28686231; PubMed Central PMCID: PMCPMC5501266.
20. World Health Organization. Global action plan on antimicrobial resistance. Geneva: World Health Organization; 2015.
21. Eurosurveillance editorial t. WHO member states adopt global action plan on antimicrobial resistance. Euro Surveill. 2015;20(21). Epub 2015/06/13. PubMed PMID: 26062562.
22. Kenyon CR, Schwartz IS. Effects of sexual network connectivity and antimicrobial drug use on antimicrobial resistance in Neisseria gonorrhoeae. Emerging infectious diseases. 2018;24(7):1195.
23. Kenyon C, Schwartz IS. A combination of high sexual network connectivity and excess antimicrobial usage induce the emergence of antimicrobial resistance in Neisseria gonorrhoeae Emerg Infect Dis. 2018;24(7). doi: https://doi.org/10.3201/eid2407.172104.
24. Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ. 2008;86(4):317-9. Epub 2008/04/29. PubMed PMID: 18438522; PubMed Central PMCID: PMCPMC2647421.
25. Kenyon C. Screening is not associated with reduced incidence of gonorrhoea or chlamydia in MSM; an ecological study of 23 European countries. F1000Res. 2019.
26. Jenness SM, Weiss KM, Goodreau SM, Gift T, Chesson H, Hoover KW, et al. Incidence of Gonorrhea and Chlamydia Following Human Immunodeficiency Virus Preexposure Prophylaxis Among Men Who Have Sex With Men: A Modeling Study. Clin Infect Dis. 2017;65(5):712-8. Epub 2017/05/16. doi: 10.1093/cid/cix439. PubMed PMID: 28505240; PubMed Central PMCID: PMCPMC5848234.
27. Hook EW, Handsfield H. Gonococcal infections in the adult In: Holmes KK, editor. Sex Transm Dis. 3rd ed. New York: McGraw-Hill, Health Professions Division; 1999. p. xxiii, 1454, 118 p.
28. Plummer FA, Simonsen JN, Chubb H, Slaney L, Kimata J, Bosire M, et al. Epidemiologic Evidence for the Development of Serovar-Specific Immunity after Gonococcal-Infection. Journal of Clinical Investigation. 1989;83(5):1472-6. doi: Doi 10.1172/Jci114040. PubMed PMID: WOS:A1989U329800005.
29. Geisler WM, Lensing SY, Press CG, Hook EW, 3rd. Spontaneous resolution of genital Chlamydia trachomatis infection in women and protection from reinfection. J Infect Dis. 2013;207(12):1850-6. doi: 10.1093/infdis/jit094. PubMed PMID: 23470847; PubMed Central PMCID: PMCPMC3654745.
30. Omori R, Chemaitelly H, Althaus CL, Abu-Raddad LJJSTI. Does infection with Chlamydia trachomatis induce long-lasting partial immunity? Insights from mathematical modelling. 2018:sextrans-2018-053543.
31. Ridpath AD, Chesson H, Marcus JL, Kirkcaldy RD, Torrone EA, Aral SO, et al. Screening Peter to Save Paul: The Population-Level Effects of Screening Men Who Have Sex With Men for Gonorrhea and Chlamydia. Sex Transm Dis. 2018;45(9):623-5. Epub 2018/07/12. doi: 10.1097/OLQ.0000000000000892. PubMed PMID: 29994935; PubMed Central PMCID: PMCPMC6086737.
32. Vanden Berghe W, Nostlinger C, Buve A, Beelaert G, Fransen K, Laga M. A venue-based HIV prevalence and behavioural study among men who have sex with men in Antwerp and Ghent, Flanders, Belgium, October 2009 to March 2010. Euro Surveill. 2011;16(28). Epub 2011/07/29. PubMed PMID: 21794222.
33. Wetenschappelijk Instituut Volksgezondheid (WIV-ISP). Epidemiology of AIDS and HIV in Belgium, 2016. Volksgezondheid & Surveillance. 2017; https://epidemio.wiv-isp.be/ID/reports.
34. Chalmet K, Staelens D, Blot S, Dinakis S, Pelgrom J, Plum J, et al. Epidemiological study of phylogenetic transmission clusters in a local HIV-1 epidemic reveals distinct differences

between subtype B and non-B infections. BMC Infect Dis. 2010;10:262. Epub 2010/09/09. doi: 10.1186/1471-2334-10-262. PubMed PMID: 20822507; PubMed Central PMCID: PMCPMC2940905.
35. Osbak K, Kenyon C. Superimposing incident STIs on HIV phylogram to investigate possible misclassification of MSM as heterosexuals in a cohort in Antwerp, Belgium. International Journal of STD & AIDS. 2019.
36. Kalghatgi S, Spina CS, Costello JC, Liesa M, Morones-Ramirez JR, Slomovic S, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med. 2013;5(192):192ra85. Epub 2013/07/05. doi: 10.1126/scitranslmed.3006055. PubMed PMID: 23825301; PubMed Central PMCID: PMCPMC3760005.
37. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007;1(1):56-66. doi: 10.1038/ismej.2007.3. PubMed PMID: 18043614.
38. Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA. Association of antibiotics in infancy with early childhood obesity. JAMA Pediatr. 2014;168(11):1063-9. Epub 2014/09/30. doi: 10.1001/jamapediatrics.2014.1539. PubMed PMID: 25265089.
39. Marra F, Marra CA, Richardson K, Lynd LD, Kozyrskyj A, Patrick DM, et al. Antibiotic Use in Children Is Associated With Increased Risk of Asthma. Pediatrics. 2009;123(3):1003-10. doi: 10.1542/peds.2008-1146. PubMed PMID: WOS:000263825500037.
40. Stewart-Brown S, Farmer AJBBMJ. Screening could seriously damage your health. 1997;314(7080):533.
41. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U S A. 2018;115(15):E3463-E70. Epub 2018/03/28. doi: 10.1073/pnas.1717295115. PubMed PMID: 29581252; PubMed Central PMCID: PMCPMC5899442.
42. Moi H, Radcliffe K, van de Laar M, Janier M, Jensen JS, Vader HM. 2009 European Guideline on the management of non-gonococcal urethritis. 2009.
43. Radcliffe K, Janier M, France DJSJ, Neumann M, Patel R, Ross J, et al. 2007 EUROPEAN GUIDELINE (IUSTI/WHO) ON THE MANAGEMENT OF PROCTITIS, PROCTOCOLITIS AND ENTERITIS CAUSED BY SEXUALLY TRANSMISSIBLE PATHOGENS.
44. Bignell C, Unemo M, European STIGEB. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS. 2013;24(2):85-92. doi: 10.1177/0956462412472837. PubMed PMID: 24400344.
45. KCE. Diagnosis and management of gonorrhoea and syphilis. https://kcefgovbe/en/diagnosis-and-management-of-gonorrhoea-and-syphilis.
46. de Vries HJ, Zingoni A, Kreuter A, Moi H, White J. 2013 European guideline on the management of lymphogranuloma venereum. Journal of the European Academy of Dermatology and Venereology. 2015;29(1):1-6.
47. De Baetselier I, Osbak KK, Smet H, Kenyon CR, Crucitti T. Take three, test one: a cross-sectional study to evaluate the molecular detection of Chlamydia trachomatis and Neisseria gonorrhoeae in pooled pharyngeal, anorectal and urine samples versus single-site testing among men who have sex with men in Belgium. Acta Clin Belg. 2018:1-5. Epub 2018/11/13. doi: 10.1080/17843286.2018.1545376. PubMed PMID: 30415606.
48. Thielemans E, Wyndham-Thomas C, Henrard S, De Vleeschouwer A, Steensels D, Montesinos I, et al. Screening for Chlamydia trachomatis and Neisseria gonorrhoeae Infections in Men Who Have Sex With Men: Diagnostic Accuracy of Nucleic Acid Amplification Test on Pooled Urine, Anorectal, and Pharyngeal Specimens. Sex Transm Dis. 2018;45(3):195-8. doi: 10.1097/Olq.0000000000000722. PubMed PMID: WOS:000425368900016.
49. Strauss A, Corbin J. Basics of qualitative research: techniques and procedures for developing grounded theory: Sage publications Thousand Oaks, CA; 2007.
50. Montano MA DJ, Barbee LA, Golden MR, Khosropour CM,. Changes in sexual behavior and STI diagnoses among MSM using PrEP in Seattle, WA. CROI 2017. 30(8):7.

51. Janier M, Hegyi V, Dupin N, Unemo M, Tiplica GS, Potocnik M, et al. 2014 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol. 2014;28(12):1581-93. doi: 10.1111/jdv.12734. PubMed PMID: 25348878.
52. Marcus U, Hickson F, Weatherburn P, Schmidt AJ, Network E. Estimating the size of the MSM populations for 38 European countries by calculating the survey-surveillance discrepancies (SSD) between self-reported new HIV diagnoses from the European MSM internet survey (EMIS) and surveillance-reported HIV diagnoses among MSM in 2009. BMC Public Health. 2013;13:919. doi: 10.1186/1471-2458-13-919. PubMed PMID: 24088198; PubMed Central PMCID: PMC3850943.
53. Kenyon CR, Osbak K. How many MSM in Europe could benefit from PrEP--a 9 billion Euro question? Int J STD AIDS. 2015;26(13):988-90. doi: 10.1177/0956462415584490. PubMed PMID: 26490178.
54. Lewis DA. The role of core groups in the emergence and dissemination of antimicrobial-resistant N gonorrhoeae. Sex Transm Infect. 2013;89 Suppl 4:iv47-51. doi: 10.1136/sextrans-2013-051020. PubMed PMID: 24243880.
55. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations: HM Government and Welcome Trust; 2018.
56. Buyze J, Vandenberghe W, Hens N, Kenyon C. Current levels of gonorrhoea screening in MSM in Belgium may have little effect on prevalence: a modelling study. Epidemiol & Infect. 2018;1:1-6. doi: 10.1017/S0950268818000092.

■ APPENDICES

1. RISK ASSESSMENT OF THE TRIAL INTERVENTION(S)

Risks associated with trial interventions
☐ A ≡ Comparable to the risk of standard medical care
☐ B ≡ Somewhat higher than the risk of standard medical care
☐ C ≡ Markedly higher than the risk of standard medical care

Justification:
As outlined more fully above in section 2, the non-screening arm may have a higher incidence of symptomatic Ng and or Ct infections. There might also be more onward transmission of Ng/Ct to partners.

What are the key risks related to therapeutic interventions you plan to monitor in this trial?		How will these risks be minimised?		
Intervention	Body system/Hazard	Activity	Frequency	Comments
Not screening	Increased symptomatic Ng/Ct infections	DSMB Monitoring	Once 50% and 100% of month 6 visits completed	
Not screening	Increased transmission of Ng/Ct	DSMB Monitoring	Once 50% and 100% of month 6 visits completed	

We plan to set up a Data and Safety Monitoring Board (DSMB) within the Trial Steering Committee (TSC) to evaluate if the non-screening arm has an unacceptably high incidence of symptomatic Ng/Ct. This DSMB will include two independent STI experts (Infectious Disease Physicians/Epidemiologists) and the study statistician who will together decide what threshold they determine to be an unacceptable risk and what methodology to use to evaluate this risk.

The DSMB will evaluate the incidence of symptomatic Ng + Ct in both arms once 50% and 100% of individuals have completed their 6 month visit. If the incidence of symptomatic Ng + Ct in the non-screening arm is more than two-fold higher than the screening arm at either of these time points, then serious consideration should be given to stopping the study

For the reasons described above, no safety reporting will be performed besides the reporting of STIs for all participants for the whole duration of the trial.

Since this trial is not considered as a clinical trial according to European Regulation, there will be no submission of the trial documents to the FAMHP.

2. AUTHORISATION OF PARTICIPATING SITES

Appendix 2.1. Required documentation

The following documents will be requested from each of the five participating sites before study initiation:

- CV of the Principal Investigator (PI)
- Final feasibility report

Appendix 2.2. Procedure for initiating/opening a new site

Before a site can start enrolment of participants, a site initiation visit (SIV) will be performed by the sponsor.

The SIV will be performed by the monitor. The monitor will preferably be accompanied by one of the sponsor researchers of the study. The sponsor's data manager will provide training on the electronic CRF database, but this task can also be performed by the study monitor. Only after the SIV for a specific site has taken place and after approval of the monitor – in agreement with the sponsor researcher(s) – the study can be started at this site.

At least the following activities will be carried out by the monitor during the SIV and documented in the SIV report:

- Ethical and GCP requirements: The monitor will meet all the site Investigators and relevant study staff to verify that they have sufficient understanding of the study protocol, procedures and the related GCP requirements.
- Protocol and Case Report Form (CRF) review: The monitor will review the protocol and the eCRF, as well as all the study SOPs, forms and other essential documents, with all the Investigators and relevant study staff.
- The participant flow and screening and recruitment strategies will be discussed so that potential bottlenecks or other problems can be identified and anticipated.
- Investigator file (IF): The monitor will provide the documents for the IF, review them with the site Principal Investigator (PI) and relevant study staff, and discuss the further management.
- Visit of site facilities: The monitor will visit the study site facilities, to confirm that they are adequate for performing key clinical, laboratory and data management activities.
- Communication: The monitor and the site Investigator(s)/site staff will agree on clear communication lines, so that the site Investigator(s)/site staff can contact the monitor and any concerned members of the TSC/TMG any time in-between two visits, for clarification or resolution of additional questions and/or ongoing problems.

Appendix 2.3. Principal Investigator responsibilities

The PI of every participating site will have the following responsibilities:

- Attend the Site Initiation Visit and make sure they are available for discussion during the Site Monitoring Visits and Close-Out Visit
- Ensure that all participating staff are adequately trained in the study protocol and study procedures and have up to date GCP and/or GCLP training as appropriate

- Ensure that the Investigator Site File (ISF) is accurately maintained
- Ensure that all important safety or trial-related information is disseminated timely to all stakeholders within their site

3. SAFETY REPORTING FLOW CHART

All episodes of symptomatic Ng/Ct will be reported as described above in the eCRF and will be discussed at after the two evaluations by the DSMB.

4. AMENDMENT HISTORY

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	2.1	28 Apr 2021	Natacha Herssens	ICF procedure for qualitative study adapted and possibility of online FGDs added. Adaptation of section 8.12 withdrawal criteria