

**Time to diagnosis of HCV re-infection with the
use of a self-test. A feasibility study.**

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Coordinating investigator/project leader	B. Rijnders, Erasmus MC, Rotterdam Erasmus MC, Room Rg-530 Postbox 2040 3000 CA Rotterdam, the Netherlands Phone No. +31107033510 Fax No. +317035945 b.rijnders@erasmusmc.nl
Principal investigator at Erasmus MC	B. Rijnders, MD PhD
Subinvestigators at Erasmus MC:	R. Verwijs, MD
Other study centers:	J. Arends, UMCU, Utrecht H. Ammerlaan, Catharina-Ziekenhuis, Eindhoven C. Delsing, Medisch Spectrum Twente, Enschede M. Claassen, Rijnstate, Arnhem J. den Hollander, Maasstad, Rotterdam R. Soetekouw, Spaarne Gasthuis, Haarlem
Sponsor (in Dutch: verrichter/opdrachtgever)	Erasmus MC, department of internal medicine, section infectious diseases
Subsidising party	Gilead sciences

Independent expert (s)	Dr. R.J. de Knegt, Erasmus MC, Room Na-607 Postbox 2040 3000 CA Rotterdam, the Netherlands Phone No. +31107033793/35942 r.deknegt@erasmusmc.nl
Laboratory sites	
Laboratory of Virology, EMC	Erasmus MC, Room Na-420 Postbox 2040 3000 CA Rotterdam, the Netherlands Phone No. +31107035514

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or legal representative: Head of Department Dr. S.C.E. Klein Nagelvoort-Schuit, MD, PhD Erasmus MC, Room RG-536 Postbox 2040 3000 CA Rotterdam, the Netherlands Phone No. +31107037549 <u>s.schuit@erasmusmc.nl</u>		
Coordinating Investigator/Project leader/Principal Investigator: Bart J.A. Rijnders, MD, PhD Erasmus MC, Room RG-530 Postbox 2040 3000 CA Rotterdam, the Netherlands Phone No. +31107033510 Fax No. +317035945 <u>b.rijnders@erasmusmc.nl</u>		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it, is not regarded as the sponsor, but is referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale:

Elimination of HCV was recently formulated as a WHO target and was set for the year 2030. Globally, approximately 6.2% of HIV-infected patients are co-infected with HCV. Of the patients living with HIV, people who inject drugs (PWID) and men who have sex with men (MSM) are at particularly high risk of HCV co-infection. However, as a result of the early adaptation of opioid substitution and needle exchange programs in the Netherlands, the number of PWID co-infected with HIV and HCV is limited. This is unfortunately not the case for HIV-infected MSM. Until recently, the prevalence of chronic HCV in Dutch HIV+MSM was very high at 4.8% (compared with 0.2% in the Dutch population in general). However, after the restrictions on the use of direct-acting antivirals against HCV (DAA) were lifted in 2015, the prevalence of chronic HCV in HIV+MSM decreased rapidly. With this decreasing prevalence of chronic HCV a decrease in the *incidence* of *acute* HCV infections in Dutch HIV+MSM was observed as well. Indeed, while the incidence of *acute* HCV in HIV+MSM in care in the Netherlands was 1.1% in 2014, this decreased by 51% in 2016. However, no further decline in the number of *acute* HCV infections was observed in 2017. Also, the incidence of HCV *re*-infections in HIV+MSM that were cured of a previous HCV infection continues to be very high in the DAA era with reported rates varying between 5-10% per year.

The continuously high *re*-infection risk and the lack of a further decline in the HCV incidence after 2016 illustrates that universal DAA therapy for all patients diagnosed with a chronic HCV infection on its own will not result in HCV elimination. Other interventions are needed to reach the WHO goal of HCV elimination by 2030. One of these additional interventions may be decreasing the time to diagnosis of HCV *re*-infections in order to decrease the duration that these *re*-infected patients may transmit their HCV to sex partners. Indeed, if the diagnosis of HCV *re*-infection is made earlier, counseling on transmission risk in combination with the prompt initiation of HCV therapy will prevent transmission to sex partners and prevent new HCV infections on the population level.

The study we describe here was designed to evaluate the effect and feasibility of more frequent and home-based testing for HCV on the time to diagnosis and treatment of HCV *re*-infections.

Objective:

To assess the effectiveness of HCV RNA self-testing in reducing the time to diagnosis of HCV *re*-infection in MSM previously cured of an HCV infection, compared to the current diagnostic standard of care.

To evaluate whether the uptake of self-testing is sufficient and warrants the use of HCV RNA self-testing in clinical practice.

Study design:

Prospective controlled intervention trial. MSM cured of an HCV infection who are at continued risk for an HCV *re*-infection (based on the results of a short questionnaire, APPENDIX B) are offered HCV RNA self-testing and asked to use the test every 6 months for 2 consecutive years.

Study population:

225 to 250 adult MSM cured of HCV from 10-15 HIV and PREP clinics in the Netherlands and Belgium.

Intervention:

Eligible patients are instructed on the use of a capillary blood self-collection kit. They receive 2 kits per year for 2 consecutive years to allow them to send plasma to the virology lab of the Erasmus MC every 6 months by regular post mail.

Primary endpoints:

Comparison of the time to HCV *re*-infection diagnosis in patients using the HCV RNA self-test (intervention) with the time to HCV *re*-infection diagnosis with the standard diagnostic approach (control) in the modified intention to treat population.

Secondary endpoints:

1. Comparison of the time to HCV *re*-infection diagnosis in patients using the HCV RNA self-test (intervention) with the time to HCV *re*-infection diagnosis with the standard diagnostic approach (control) *in the subpopulation that has sent in all planned self-tests during their entire follow-up* (per protocol analysis).
2. Of the HIV+MSM that were offered to participate in the study, the percentage that accepted to participate *and* eventually self-collected and sent in at least one plasma sample in each 12-month period of study participation.

3. Overall incidence of HCV *re*-infection in the entire study population regardless of the type HCV diagnostic test that was used.

4. Number of screen failures as a result of a positive HCV-RNA test at the screening visit.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The burden associated with participation in the study consists of taking a finger prick blood sample for the self-test 4 times in 2 years and sending the sample to the laboratory by regular post mail. No costs will have to be made for mailing the sample. Capillary finger-prick blood sampling is used as a standard diagnostic test for many diseases (e.g. glucose monitoring in diabetes) and is associated with a negligible risk. The study may potentially be beneficial for those participants in which an HCV re-infection is diagnosed as they will be referred for counselling and HCV therapy which has the potential to prevent transmission to sex partners.

1. INTRODUCTION AND RATIONALE

Viral hepatitis was the seventh leading cause of death worldwide in 2013, increasing from the tenth leading cause in 2010 (1). The vast majority of morbidity and mortality attributable to viral hepatitis is due to hepatitis C virus (HCV) and hepatitis B Virus (HBV). In response to this increasing public health challenge, the World Health Organization recently released targets for HBV and HCV elimination by 2030 (2). Elimination is traditionally defined as a reduction to zero in the incidence of disease in a specific population or geographical location, with continued prevention efforts required to prevent the re-establishment of transmission (3).

Because a strict definition of elimination would require substantial economic and political resources and could be unattainable in most settings, the goal of “elimination” is often flexibly defined. The recent WHO elimination “as a public health threat” targets are comprised of a 90% relative reduction in new infections and a 65% relative reduction in hepatitis-related mortality by 2030. Hence, to achieve the WHO incidence elimination targets, efforts must focus on both prevention of disease and prevention of transmission. The advent of highly effective HCV direct acting antiviral (DAA) therapy, with cure rates (also called sustained viral response, SVR) of 95% or higher has renewed optimism that substantial reductions or elimination of end stage liver disease and HCV-related mortality is a possibility. The WHO strategy does not include a discussion relating to elimination of viral hepatitis among HIV-infected populations specifically but given the shared transmission routes many people living with HIV are coinfected with viral hepatitis as well. Globally, approximately 6.2% (3.4% to 11.9%) of HIV-infected (HIV+) patients are also coinfected with HCV, equating to approximately 2.28 million (IQR 1.27 to 4.42) HIV/HCV coinfected individuals (4). The burden of HIV/HCV coinfection is particularly high in people who inject drugs (PWID) and among men who have sex with men (MSM). However, in the Netherlands, the early adoption of opioid substitution and needle exchange programs has limited the number of PWID coinfected with HIV and HCV.

Regarding the epidemiology of HCV in HIV+MSM, it has been estimated that globally 6.4% of them are coinfected with HCV (4). Indeed, in contrast to the lack of sexual HCV transmission among heterosexual couples, HCV transmission readily occurs among MSM during anal sex. Therefore, it comes as no surprise that before restrictions on the use of DAA therapy were lifted in the Netherlands, the prevalence of HCV in Dutch HIV+MSM was equally high at 4.8% (5). Furthermore, not only the prevalence, but also the incidence of HCV in Dutch HIV+MSM is substantial. In 2014, 99 of the 8849 or 1.1% of HIV+MSM in care in the 19 largest HIV centers spread across the Netherlands, were diagnosed (5). This high

prevalence *and* incidence is in sharp contrast with the very low overall prevalence of HCV in the Dutch population in general (0.2%), which is one of the lowest worldwide (6, 7). Therefore, if the Netherlands wants to achieve the WHO hepatitis elimination goals, it is opportune to aim for HCV elimination in population subgroups with a high HCV prevalence rather than the entire Dutch population. Because the large majority of the HIV infected MSM in the Netherlands is aware of their HIV infection and in care in one of the Dutch HIV treatment centers, this specific HCV risk group is already identified. Also, 98% of them consented to have their data used for research purposes by the Stichting HIV monitoring (SHM) as part of the ATHENA cohort. Therefore, reliable and up-to-date data on the number of HIV+MSM ever diagnosed with or treated for HCV are available, and these data are truly representative of the entire Dutch HIV+MSM population. The importance of the SHM data registry regarding the HCV epidemiology in HIV+ patients is well illustrated by several recent studies. In 2018, Boerekamps et al. used SHM data to measure the impact of the introduction of unrestricted DAA therapy in the Netherlands as of November 2015 on the prevalence of HCV among HIV+ patients (8). The study showed that just 15 months after the reimbursement restrictions on DAA therapy were lifted, already 82% of HCV coinfected patients were cured or receiving DAA therapy. In MSM this was even higher at 89%. Furthermore, because patients cured of HCV can no longer transmit HCV to their partner, it was anticipated that this rapid treatment uptake would result in a decrease in new (incident) HCV infections as well. This was indeed observed as the rapid uptake of DAA therapy in HIV+MSM was followed by a 51% decrease in new (=acute) HCV infections in 2016 (9). These interesting observations have been welcomed with enthusiasm in the HIV-HCV coinfection research community as an example of “HCV microelimination” and an exemplary first step towards HCV elimination in general (10, 11).

However, not all observations regarding HCV elimination in HIV+MSM have been positive. Furthermore, it is uncertain to what extent the decline in the incidence of acute HCV that was observed is caused by the decreasing prevalence of HCV. Although we have no reasons to believe that risk behavior has improved, other factors may have played a role as well. Also, although the 51% lower HCV incidence seen in 2016 was also observed in 2017, no further decline in the incidence was observed (Boerekamps, personal communication). Several observations may explain this. First, HCV transmission among MSM doesn't stop at the country borders. Indeed, cross-border HCV transmission networks have been well described (12). Because the DAA treatment uptake is less pronounced in some other European countries, cross-border transmission may become more important and may hamper HCV elimination. Although the reimbursement restrictions that explain this lack of treatment uptake have been lifted in some European countries in 2017 (Switzerland, Spain, Italy, Portugal)

they still remain in place in others in 2018 (e.g. in the U.K. and Belgium). Another important hurdle that needs to be addressed is the number of HCV *re*-infections that is observed. Indeed, several studies have shown that risk of an HCV *re*-infection in HIV+MSM previously cured of an HCV infection is extremely high in the first 1 to 3 years after their infection was cured at 5-10% per year(13-15). Therefore, a subgroup of HIV+MSM at very high risk of HCV *re*-infection probably continues to fuel the epidemic. Last but not least, in contrast to what was believed previously, sexual transmission of HCV is not limited to HIV+MSM. Indeed, several recent publications describe a similarly high incidence of HCV in specific subgroups of HIV uninfected MSM (16-18). Indeed, now that it has been convincingly shown that HIV infected patients do not transmit HIV to their sex partner when their HIV viral load is undetectable as a result of combination antiretroviral therapy, the risk of acquiring HIV is no longer a reason to avoid sex with an HIV-infected partner. Furthermore, HIV uninfected MSM can protect themselves against HIV with HIV pre-exposure prophylaxis and this will increase mixing between HIV+ and HIV- MSM as well.

For all the reasons described above, we consider it unlikely that, even in HIV+ MSM in the Netherlands, HCV elimination will be possible if no additional interventions are implemented. Therefore, we designed 3 studies of which the common goal is to provide policy makers, physicians and patients with data that can help in choosing the most effective interventions needed to eliminate HCV in men who have sex with men.

The 3 studies consist of

1. A phylogenetic study; By sequencing a large number of viruses from patients diagnosed with a prevalent or incident HCV infection, we hope to get a better picture on current transmission networks in order to identify the source of incident HCV infections in MSM in Dutch patients. This will be a study in collaboration with the Academic Medical Center in Amsterdam and HCV researchers from outside the Netherlands.
2. An HCV prevalence study; This study will measure the prevalence of undiagnosed HCV in HIV *negative* MSM visiting the sexually transmitted diseases GGD clinic in order to evaluate the usefulness of systematic screening for HCV of HIV negative MSM attending STD clinics in the Netherlands.
3. An HCV *re*-infection study; In this study we will evaluate if HCV RNA self-testing can significantly decrease the time to diagnosis of an HCV *re*-infection. The study will include MSM previously cured of an HCV. If a substantial decrease in the time to diagnosis and

therefore also the time to “awareness” and the time to the initiation of HCV therapy and cure is achievable, this will limit onward transmission and prevent new HCV infections. This study is described below.

INTRODUCTION TO STUDY NR.3.

As previously mentioned, the HCV *re*-infection rate in HIV+MSM is very high due to ongoing risk behavior. In a prospective case-control study, Vanhommerig et al. identified several risk factors for acquiring an HCV infection. These were receptive unprotected anal intercourse, sharing sex toys, unprotected fisting, injecting drugs during sex, sharing straws when snorting drugs, having a lower CD4 cell count and having a recent diagnosis of ulcerative sexually transmitted infection (19). Various studies report on *re*-infections following SVR in HIV+MSM, but the incidence estimates have a considerable range, which could be explained by differences in study populations regarding risk behaviors, harm reduction interventions and background viremic prevalence (15). In a retrospective study in 606 previously HCV infected HIV+MSM from Austria, France, Germany and the UK, a *re*-infection incidence of 7.3/100 person-years was reported (14). In a smaller Dutch study from the pre-DAA era, Lambers et al. found a much higher HCV *re*-infection incidence of 15.2/100 person-years in 56 HIV+MSM in the Netherlands (20). Consequently, it is clear that HIV+MSM with a previously cured HCV infection have an increased risk of acquiring a new HCV infection. At the moment, HIV+MSM with a previously cured HCV infection undergo ALT testing approximately twice a year during their regular HIV outpatient clinic visit. If an ALT elevation is observed an HCV RNA test is performed to exclude or confirm a new HCV infection. However, in acute and chronic HCV infections ALT is not always elevated. ALT levels correlate with the duration of HCV infection, the viral load and the presence of periportal bridging/necrosis and it has been estimated that up to 25% (range 10 to 40%) of patients with chronic hepatitis C infection have persistently normal aminotransferase levels (21). Given these factors, using an HCV RNA as well as increasing the frequency of ALT testing both have the potential to shorten the time to diagnosis of an HCV *re*-infection significantly. If this is indeed the case, a shorter time to diagnosis (and treatment) will also reduce the time that a patient is at risk of transmitting HCV to others. Therefore, it may be one of the additional steps that is needed towards the elimination of HCV.

2. OBJECTIVES

The main objective of this study is to demonstrate that the use of a HCV RNA self-test will be an effective and feasible way to decrease the time to diagnosis of an HCV *re*-infection. If this is indeed the case, interventions to prevent HCV transmission to sex partners (e.g. counseling, initiation of DAA treatment) can be initiated sooner after an HCV *re*-infection.

For this purpose, patients will be offered to be trained in capillary blood sampling and separation of plasma at home and will send the plasma sample, by regular post mail, to a central lab for HCV RNA testing. The HCV RNA self-test will be done twice yearly on top of the standard of care ALT testing policy that is also done twice a year at the HIV outpatient clinic. Therefore, patients in the study will undergo HCV testing 4 times a year; twice a year with ALT testing during their regular HIV outpatient clinic visit (followed by an HCV RNA test if the ALT turns out to be elevated) and twice a year with the home-based HCV RNA test. This self-test is done in between the HIV outpatient clinic visits (figure 1).

Primary objective

1. Measure if and to what extent HCV RNA self-testing decreases the time from HCV re-infection to diagnosis compared with the current standard of care.

Secondary objectives:

1. Measure the uptake and acceptability of HCV RNA self-testing in MSM cured of an HCV.
2. Measure the incidence of HCV re-infection in MSM in the Netherlands and Belgium in 2018-2019-2020.

3. STUDY DESIGN

Single arm open label multicenter study.

We consider a single arm open label multicenter study the most appropriate design, given the relatively small patient population at risk for HCV re-infection in the Netherlands. Indeed, the number of HIV+MSM that fulfill the in- and exclusion criteria (see below) in the participating Dutch study centers is estimated at 250-350, of which a subset will be interested in study participation. Three additional Belgian study centers and one Swiss center have also agreed to participate in the study. Even with the participation of these foreign HIV treatment centers, a randomized fully powered study remains unachievable. Indeed, if the time to diagnosis would be reduced from 4.5 to 2.5 months with the intervention and assuming a standard deviation of 2 and an 80% power and alfa of 5%, the study would need to include 2 groups of patients in which a minimum of 16 re-infections would be diagnosed in each group (see sample size calculation below). With an incidence of HCV re-infections of $\pm 8\%$ during the 2 years of follow-up this would mean that 2×200 patients need to be included to diagnose 2×16 re-infections. As our most optimistic inclusion scenario will allow recruitment of 250

patients in 2 years, this means that a single arm study with 200 patients will already take 4 years to complete given the follow-up time of 2 years of each of the patients. Therefore, we consider a single arm open label multicenter study the most appropriate.

For the purpose of the analysis, we will use the study population as its own “virtual” control group (see below for a more detailed explanation of this control group).

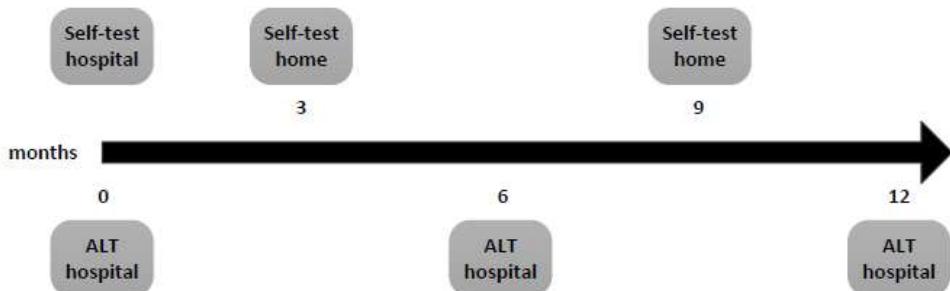
Intervention:

The self-test that is used to detect HCV RNA is performed every 6 months on top of standard of care ALT test as described above. The HCV RNA testing will be done at home by the patient 3 months after each HIV outpatient clinic visit and sends the plasma sample to our laboratory by regular post mail. If HCV RNA is detected, the treating physician will be informed and the patient contacted immediately and invited to visit the outpatient clinic for confirmation of the positive HCV RNA self-test and evaluation of liver enzymes. If the presence of HCV RNA is confirmed, HCV therapy can then be initiated according to the Dutch or Belgian HCV treatment guideline.

Standard HCV diagnostic procedures in the “virtual control group”:

The standard of care HCV re-infection diagnostic strategy used by HIV outpatient clinics is measuring ALT levels every 6-months. If an ALT elevation is documented, HCV RNA testing is performed to diagnose a potential HCV re-infection. If this HCV RNA test is positive, HCV therapy can then be initiated according to the Dutch HCV treatment guideline.

Figure 1 illustrates the timing of the home-based HCV RNA test in the intervention group; The self-test is done every 6 months in between the ALT tests at the outpatient HIV clinic. Furthermore, the self-test is done at the screening visit as well to exclude that patients with an undiagnosed HCV infection are included.

Figure 1.

Defining the time to diagnosis in the intervention and the virtual control group:

Patients diagnosed with an HCV re-infection by the HCV RNA self-test are contacted immediately by the site investigator and invited to visit the outpatient clinic to have their liver enzymes tested and to confirm the HCV diagnosis with a whole blood HCV RNA PCR test. If at that time the ALT level is elevated, we will assume that an HCV RNA test would have been done by the treating physician at the next 6-monthly HIV outpatient clinic visit (in \pm 3 months) and therefore the HCV diagnosis would have been made at that time point.

In patients diagnosed with an HCV re-infection in which the ALT level turns out to be normal, we will assume that the ALT level would *not be elevated* at the next planned HIV outpatient visit (in general \pm 3 months later), but that it *would be elevated* at the consecutive HIV outpatient clinic visit (in general \pm 9 months later) and therefore the HCV diagnosis would have been made at that time point. This conservative approach that assumes an ALT elevation in all patients 9 months after the positive HCV RNA test is needed because we anticipate that all patients will have started HCV therapy within 6 months after diagnosis and this leads to prompt ALT normalization. Therefore, we cannot use the actual measured ALT level 9 months after the positive HCV RNA test.

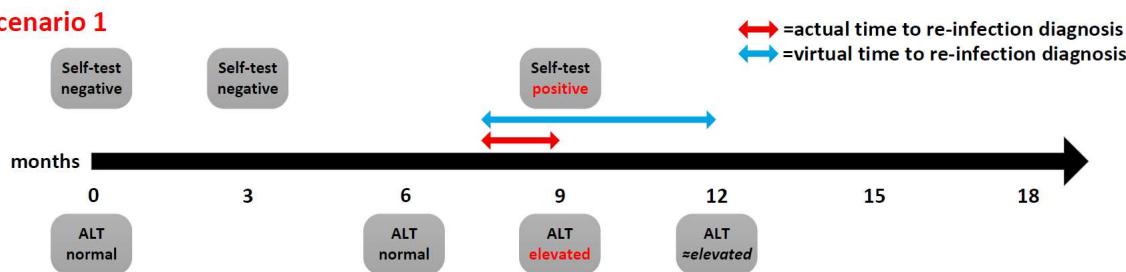
The date of infection is estimated as the midpoint between the date of last negative HCV test and the date of the first positive HCV test. The last negative HCV test can be either a negative ALT test or a negative HCV RNA test. The first positive HCV test can be either a positive HCV RNA self-test or a positive whole blood HCV RNA test performed in the hospital. This estimation of the date of infection will be used for the analysis of the time to diagnosis in both the intervention and the virtual control group.

This is illustrated by the following example and in figure 2. A study patient with a positive HCV RNA self-test 9 months after the start of the study had an ALT within the normal range at 6 months. When he is informed about the positive self-test and the positive test is confirmed by the local investigator his ALT is measured as well and turns out to be elevated. For this patient, the date of infection is estimated at 7.5 months ($6+((9-6)/2)$). The actual time to re-infection diagnosis in the intervention arm is (9-7.5) or 1.5 month. To evaluate the time to re-infection diagnosis as part of the “virtual” control group, we will disregard the result of the self-test at 9 months and assume that at 12 months an elevated ALT would have been detected, because his ALT was also elevated at 9 months. Therefore, the virtual time to re-infection diagnosis for this patient is (12-7.5) or 4.5 months (scenario 1 in figure 2). However, if the ALT level of this patient would have been normal at 9 months, the virtual time to re-infection diagnosis would be different. Indeed, while the actual time to re-infection diagnosis would still be 1.5 month, the virtual time to re-infection diagnosis would be (18-7.5) and therefore 10.5 months because we will assume that ALT would still be normal at the 12 months HIV outpatient clinic visit but would have become elevated at the next consecutive HIV outpatient clinic visit at 18 months (scenario 2 in figure 2).

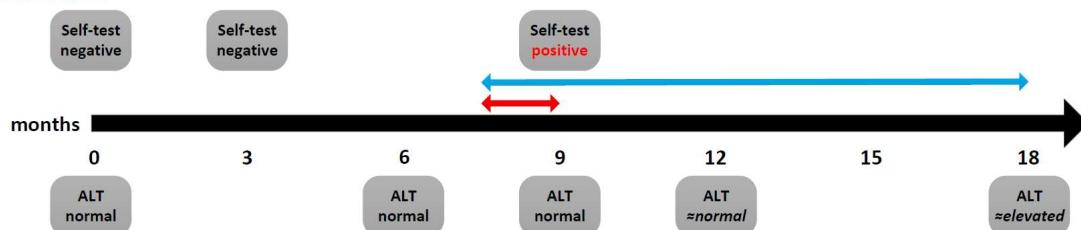
Please note that all patients will undergo an HCV RNA self-test at the time of screening for the study. This will not only allow for the demonstration of the self-test by the trial-nurse but will also ascertain that patients with an undiagnosed chronic HCV are not included.

Figure 2

Scenario 1



Scenario 2



4. STUDY POPULATION

4.1 Population (base)

Study population:

Only patients that provide written informed consent will be included. They will first have to review the instruction video that explains and shows how the capillary blood collection and separation of plasma should be done. If the patient is still willing to participate after he has seen the instruction video and received all information by the research team, he will receive the self-testing kit with all materials needed to send in 4 plasma samples over a period of 2 years. The patients will be offered HCV RNA self-testing for 2 consecutive years and thus during 400 patient years of follow-up. Recruitment will stop when at least 1 plasma sample is received from 200 patients. Therefore, we estimate that we will need to include 225 to 250 patients to have an evaluable population of at least 200.

We allow HIV+ as well as HIV- MSM to be included as long as they fulfill the in- and exclusion criteria. HIV negative MSM can only be included if they are taking PREP and if HCV RNA testing is not done as the standard of care HCV diagnostic strategy at the PREP clinic but ALT testing is done as an HCV testing policy no more often than 3x/years. Twice yearly testing is the standard of care in HIV+ patients in the Netherlands but the frequency may be higher or lower in HIV uninfected PREP users per local policy (e.g. in Belgium).

The total number of patients that was informed about the study and the number of patients that reviewed the video but who never actually started using the HCV RNA self-test will be used to evaluate the acceptability of HCV RNA self-testing.

For the statistical analysis the following populations are defined:

Modified Intention to treat population (MITT):

All patients who signed the informed consent, received the HCV RNA self-test kit and in which the HCV RNA test at the screening visit was negative.

Per protocol population (PP):

All patients who signed the informed consent, received the HCV RNA self-test kit and in which the HCV RNA test at the screening visit was negative and who collected all 6 monthly plasma samples during the entire follow-up.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Cured of HCV defined as an SVR (=documented negative HCV RNA test) at least 12 weeks after the end of DAA therapy *and* no new documented positive HCV RNA test after the date of the SVR

OR

Spontaneous clearance of HCV infection defined as two consecutive negative HCV RNA tests at least 3 months apart after a positive HCV RNA test.

- In care for an HIV infection in an HIV clinic in a study center or HIV negative and receiving PrEP at a PrEP clinic
- Able and willing to perform the self-test at home after viewing the instruction video
- Willing to fill out a questionnaire on risk behavior at the time of HCV self-testing
- At risk of HCV re-infection according to a short questionnaire, in other words, patients should have one of the following risk factors (19):
 - Receptive unprotected (condomless) anal intercourse in the last 6 months
 - Fisting or being fisted without gloves in the last 6 months
 - Sharing toys in the last 6 months
 - Syphilis or LGV in the last 12 months,
 - Slamming (injecting drug use) in the last 12 months
 - Sharing sniffing straws or other objects to sniff drugs in the last 12 months

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age < 18
- Patients that are tested by HCV RNA as a standard of care test (e.g. in the context of PREP use) > 1x/year
- Patients that are expected to be tested by ALT at their HIV or PREP clinic <1x/year

4.4 Sample size calculation

200 HIV+MSM with a previously cured or a spontaneously resolved HCV infection will be included. These patients will be offered HCV RNA self-testing for 2 years and thus during at least 400 patient years of follow-up.

Previous reports on the incidence of HCV re-infection have observed a re-infection incidence of 5 to 15% per year. However, we should also consider that after the roll-out of DAA therapy in the Netherlands a 51% decrease in the number of acute HCV infections was observed in 2016 compared with 2014. Therefore, we estimate that the incidence of re-infection will be 4% rather than 5-15% per year. Therefore, we anticipate to diagnose a HCV re-infection in 8% of the patients during the 2 years of follow-up. With the inclusion of 200 patients and an average follow-up of 2 years per patient, we should therefore be able to diagnose 16 re-infections. The calculator for a continuous outcome superiority trial of the website sealedenvelope.com was used for the sample size calculation because the outcome we measure is continuous (days).. Now assuming a time from re-infection to diagnosis of 4.5 months or 135 days (SD of 2 months or 60 days) with the current standard of care ALT testing strategy (virtual control group) and a reduction to 2.5 months or 75 days with the HCV RNA self-testing intervention, the required sample size is 16 infections per arm. Because these 16 infections will be used to calculate the time to diagnosis for the intervention as well as for the control group, a sample size of only 16 infections will give the study 80% power to show a difference between the intervention and the virtual control group.

5. TREATMENT OF SUBJECTS

N/A

6. INVESTIGATIONAL PRODUCT

N/A

7. NON-INVESTIGATIONAL PRODUCT

N/A

8. METHODS

8.1 Study parameters/endpoints

Primary endpoints:

1. Comparison of the time to HCV *re*-infection diagnosis in patients using the HCV RNA self-test (intervention) with the time to HCV *re*-infection diagnosis with the standard diagnostic approach (virtual control) in the modified intention to treat (MITT) population.

Secondary endpoints:

1. Comparison of the time to HCV *re*-infection diagnosis in patients using the HCV RNA self-test (intervention) with the time to HCV *re*-infection diagnosis with the standard diagnostic approach (control) *in the subpopulation that sent in all planned self-tests during their entire follow-up* (Per Protocol analysis).
2. Of the HIV+MSM that were offered to participate in the study, the percentage that accepted to participate *and* eventually self-collected and sent in at least one plasma sample in each 12-month period of study participation.
3. Overall incidence of HCV *re*-infection in the entire study population regardless of the type of HCV diagnostic test that was used.
4. Number of newly diagnosed HCV infections at the time of the screening visit as a result of a positive HCV-RNA test at the screening visit.

8.2 Randomisation, blinding and treatment allocation

N/A

8.3 Study procedures

Only patients that provide written informed consent will be included. Next, they will be screened for the study which includes answering a 1-page questionnaire on sexual practices in the last 12 months (APPENDIX B)

If the patient fulfills all the in- and exclusion criteria, he will have to review the instruction video as a next step. This video explains and shows how the capillary blood collection and

plasma separation should be done. If the patient is still willing to participate after he has seen the instruction video and received all information by the research team, he will receive the self-testing kit with all materials needed to send in 4 plasma samples over a period of 2 years.

HCV RNA self-test

Patients will be instructed on how to collect capillary blood using the Demecal set, a CE-marked and dedicated tube with an incorporated sponge, apply the filter that separates the plasma and send the sample to the virology lab where the plasma will be tested with the regular HCV PCR essay. An illustration of the procedure can be reviewed here: <http://www.i-thatsme.nl/i-my-personal-soa-check/nl/videohandleiding>.

The result of the HCV RNA self-test will be communicated to the investigator of the study site who will communicate the result to the patient.

Patients will be asked to fill out a short questionnaire on sexual practices in the 6 months preceding the self-test (APPENDIX C)

The self-testing kit will include a termination letter. The patient is asked to send this letter to the study coordinator when he decides to stop using the HCV RNA self-test and fill out the reason why he has decided to do so (appendix A).

Table 12. Study procedures

Procedures at time points	Screening	Mo3	Mo6	Mo9	Mo12	Mo15	Mo18	Mo21	Mo24
Eligibility check	x								
Informed consent	x								
Screening questionnaire	x								
Instruction video	x	x		x		x		x	
Study self-test dispensing	x								
Self-test at hospital	x								

ALT measurement	x		x		x		x		x
Self-test at home		x		x		x		x	
Self-test questionnaire		x		x		x		x	
Whole blood HCV RNA PCR		x*		x*		x*		x*	

* These tests will only be performed if the result of the HCV RNA self-test is positive. The patient will be called and sent to the clinic for consecutive whole blood HCV RNA PCR (for confirmation of diagnosis) and liver enzyme testing.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Patients in which a HCV re-infection was diagnosed will leave the study.

8.5 Replacement of individual subjects after withdrawal

Patients who are withdrawn are not replaced by new subjects. However, the study will include a sufficient number of patients to have at least 200 evaluable patients (see 3.1 above)

8.6 Follow-up of subjects withdrawn from treatment

The follow-up of patients withdrawn from the study will be done by their treating physician.

8.7 Premature termination of the study

N/A

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject

health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Because the only intervention that is performed is the HCV RNA self-test (4 capillary blood samples in 2 years time), we will only report SAE that are possibly directly related to the finger prick sampling (e.g. wound infection for which a hospital admission is required).

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a

period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.3 Annual safety report

NOT APPLICABLE

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

10. STATISTICAL ANALYSIS

For the primary endpoint the time to HCV re-infection diagnosis will be compared between the intervention group ("The self-testing strategy") and the virtual control group ("The standard of care ALT-based strategy"). Descriptive statistics will be used to quantify the difference in time to diagnosis between these groups. The focus of this study is the feasibility of the diagnostics and therefore to show that the amount of reduction in time to diagnose that the test will lead to is sufficient (i.e. at least 2 months, see paragraph 4.4) to be considered clinically relevant. Since the actual time to diagnosis and the virtual time to diagnosis are measured and estimated for the same patient, these data are dependent. Consequently, for inferential statistics, the Wilcoxon signed rank test will be used in which the virtual time to diagnosis is considered the pre-intervention measurement and the actual time to diagnosis as observed with the self-test intervention will be considered the post-intervention measurement.

The time to diagnosis is defined as the number of days between the calculated time of transmission and the first positive HCV RNA test.

The calculated time of transmission is defined as the midpoint between the date of the last negative and first positive HCV test. For this purpose, the last negative HCV test can consist of a normal ALT level or a negative HCV RNA test.

The analysis of the primary endpoint will be done according to the intention to treat approach and will therefore include the MITT population as described above.

We refer to p.17 for a detailed explanation on how to define the date of the first positive HCV test in the virtual control group.

The analysis of secondary endpoint 1 is similar to the methodology described for the primary endpoint. For the secondary endpoints 2 to 4 descriptive statistics will be used and 95% confidence intervals will be reported.

For secondary endpoint 1, the follow-up time in months will be considered 24 months in all patients that did not send in the termination letter. In patients that confirmed that they stopped using the HCV RNA self-test because they no longer considered themselves at risk for HCV, the follow-up time ends on the termination date mentioned on the termination letter (or the termination date otherwise communicated by the patient as long as this date is documented in the patient file by the principal investigator and the reason to stop was that the patient considered himself no longer at risk for HCV).

10.1 Interim analysis (if applicable)

Not applicable

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be performed in accordance with the protocol, the guidelines of Good Clinical Practice/ICH, which underwrites the principles of the Declaration of Helsinki, as most recently revised by the 64th WMA General Assembly in Fortaleza, Brazil, October 2013.

11.2 Recruitment and consent

Patients will be recruited at the study sites mentioned on page 2-3. It is the responsibility of the investigators or the co-investigators to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated, and potential hazards of the study (patient information form).

Besides the specific information regarding the study, the following standard items are covered in the patient information form (Dutch: patiënten informatie formulier):

- Patient's right to withdraw from the clinical study anytime without giving reasons and without any consequences for further medical treatment.
- The information that all study findings will be stored in a computer database and handled confidentially
- Patient names will be kept separate from research data and patients will be identifiable by subject number only.
- Information about the possibility of inspection of relevant parts of the hospital records by regulatory authorities. Inspection will only take place if a confidentiality agreement has been signed.
- The existence of patient insurance policy in case the patient will be harmed by participating in the study (using the study drug)
- All novel clinically relevant information that will become available during the study and is possibly important for the patient will be communicated to him/her by one of the investigators.

The signature of an investigator or co-investigator on the form will attest that the information in the consent form was accurately explained and understood. Thereafter the patient will sign after a period of reflection. If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated after approval by the ethical committee. Then, all subjects (including those already being treated) will be informed of the new information, will be given a copy of the revised form and will be asked to give their consent to continue the study.

11.3 Objection by minors or incapacitated subjects (if applicable)

N/A

11.4 Benefits and risks assessment, group relatedness

Doing extra diagnostics can be a burden to the patient, for example due to anxiety or stress for the test results. But we believe that if a patient, after giving informed consent has decided to do the self-testing for HCV, this gives the patient not only more responsibility but also more control over his personal health and could also reduce anxiety. In addition, earlier diagnosis can make earlier treatment possible and can prevent infections to other (sexual) partners, which is of benefit to the society.

11.5 Compensation for injury**Liability insurance sponsor/investigator**

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

Insurance for study participants

The ethics committee waived the need for a WMO insurance considering the negligible risk for study participants.

11.6 Incentives (if applicable)

No incentives.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION**12.1 Handling and storage of data and documents**

Data will be handled confidential and if possible anonymously. Where it is necessary to be able to trace data to an individual subject, a subject identification code list will be used to link the data to the subject. The code will not be based on the patient initials and birth-date. The key to the code will be safeguarded by the investigator. as the data and human material will be kept for a longer period of time. The handling of personal data will comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG, UAVG).

Case record form (CRF)

All data of patients, including results from standard procedures during treatment, collected during the study will be recorded in Case Record Forms. The CRF must be completed fully and legibly. Corrections of possibly erroneous entries must be carried out in such a manner that the initial entry is not rendered illegible. Corrections should be written alongside or above the pertinent place with the date and initials. Correction fluid must not be used.

The investigators are responsible for the quality of the data recorded in the Case Record Forms (CRF). Where the investigators have not been responsible for completing the CRF, an additional signature from the co-investigator overseeing the data entry of the study must be obtained.

In the event that the investigators need to deviate from the protocol, the nature of and reasons for protocol deviation must be recorded in the hospital patient record and in the

CRF. In nearly all cases it is desirable that the patient continues the study to allow the most informative intention-to-treat analysis; however, the patient may be excluded from the per-protocol analysis.

OpenClinica or another GCP compliant system that Erasmus MC supports will be used as the eCRF.

Privacy rules

Patients will be identified in the CRF by their identification code. The investigators will keep a patient identification log, including sufficient information to link the hospital record and CRFs.

The subjects will be informed that the data will be stored on paper and electronically, that local regulations for the handling of computerized data will be followed as described in the written patient information / consent form and that identification of individual patient data will only be possible for the investigators. Furthermore, the subjects will be informed about the possibility of inspections of relevant parts of the hospital records by health authorities. These officials will be identified and have signed a confidentiality agreement. The data are stored and processed using a database program for personal computers. From this database the data will be transferred to a statistical program for further analysis. Only data, with coded patient identity will be transferred to the statistician for analysis.

Data processing

After a visual plausibility check the CRF data will be entered in the computer and processed using a database program. Data base print outs will be produced and checked by one of the principle investigators or his co-investigators. When approved, the data will be transferred from the database to a statistical data file, with conversion in uniform data and formation of a master database for further analysis. The data transfer to the statistician can take place during the study

Data achieving

Patient identification log, hospital records, informed consent forms, case record forms and databases must be kept for at least 5 years after completing the study (EU-directive 2005/28/EG). If the investigators move or retire, they must nominate someone in writing to be responsible for record keeping. Archived data may be held on microfiche or

electronic record, provided that a backup exists and a hard copy can be obtained from it if required.

12.2 Monitoring and Quality Assurance

Please refer to our monitoring plan.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final

study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

The sponsor is free to publically disclose and publish all research data. Please refer to the contract between the sponsor and the subsidising party for arrangements made concerning public disclosure and publication of research data.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Capillary blood collection for HCV RNA self-test—drops of capillary whole blood collected from a simple prick of the finger using a tube with sponge—provide a minimally invasive alternative to venous blood sampling that facilitates the collection of blood samples in home-based settings and can be done by non-medically trained personnel. It is comparable to a skin-prick test that patients with diabetes use. The risks are therefore negligible.

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