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Innovative testing and prevention strategies in the Netherlands

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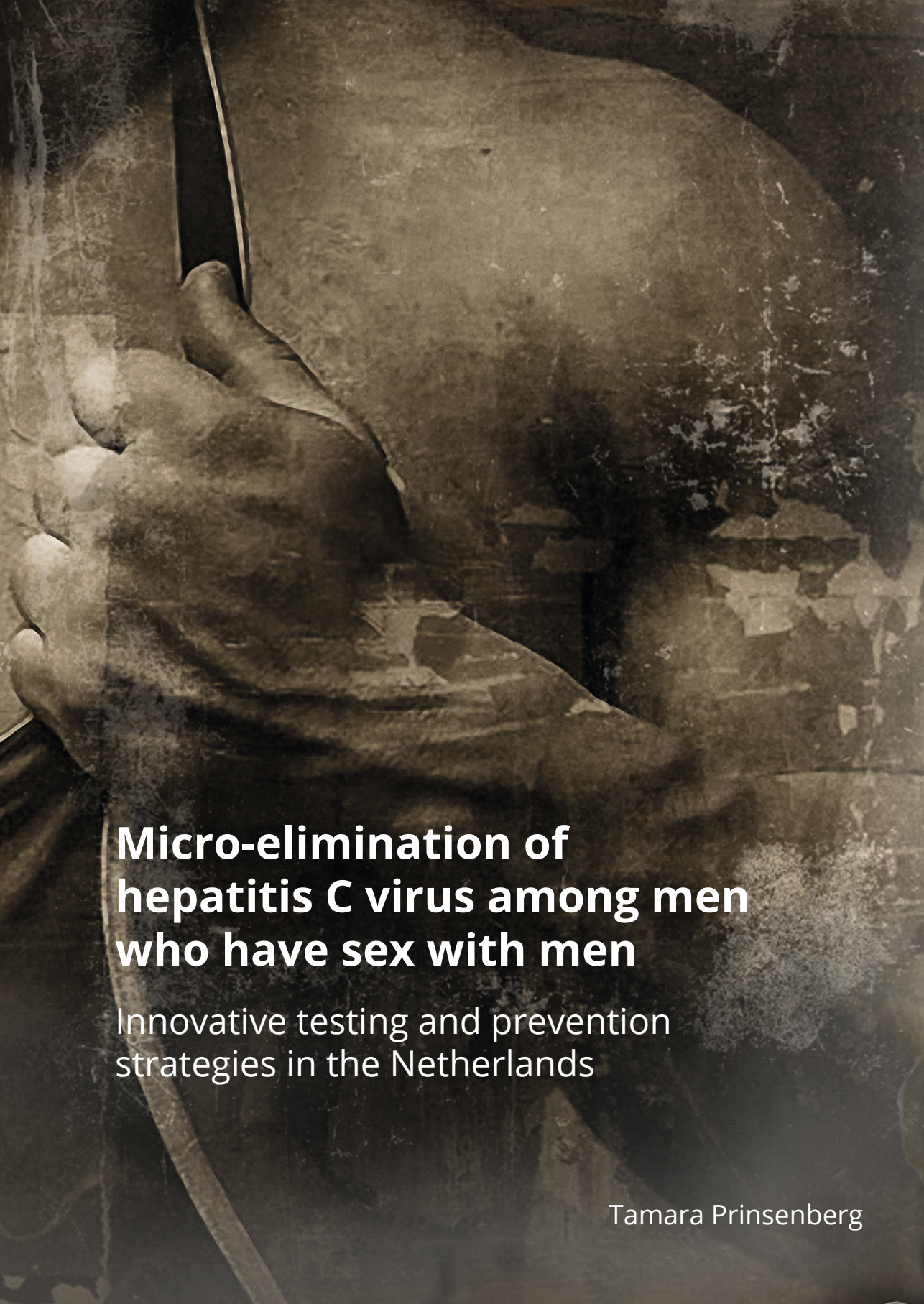

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Tamara Prinsenberg

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Chapter 1

General introduction

This PhD thesis has been completed as part of a project to develop, implement and evaluate a multilevel intervention to reduce hepatitis C virus (HCV) transmission among men who have sex with men (MSM) in Amsterdam. The project was initiated by the Amsterdam MSM Hepatitis C Free (MC Free) consortium, a partnership between the Public Health Service of Amsterdam (PHSA), Amsterdam University Medical Centers, location Academic Medical Center, Soa Aids Nederland, and Amsterdam Institute for Global Health and Development (AIGHD). The main goal of the consortium was to reduce HCV incidence among MSM in Amsterdam. With sensitive HCV tests, effective HCV treatment, knowledge about HCV transmission routes and risk reduction behaviors, all key components were available to succeed in reducing HCV transmission among MSM and subsequently achieve micro-elimination. The subsequent development of the interventions that targeted the individual, community, professional, context, patient and network levels are innovative. As well as the involvement of health professionals, gay community members, commercial stakeholders, and stakeholders from the gay community in the development process.

Hepatitis C virus

The discovery of the hepatitis C virus (HCV) started in 1972, when a novel type of hepatitis was found in patients who received blood transfusions [1]. In these patients hepatitis A virus (HAV) and hepatitis B virus (HBV) were not detected, and from 1975 onwards the term non-A, non-B hepatitis was introduced [2, 3]. After the structure of this novel RNA virus was unraveled in 1989, the name was changed to HCV [4]. HCV is an enveloped, single-stranded RNA virus, belonging to the Flaviviridae family. As HCV is a blood-borne virus, it is typically transmitted via percutaneous exposure to infected blood. HCV has a high degree of genetic diversity, and is currently classified into eight genotypes (indicated by numbers) [5] and more than a hundred subtypes (indicated by letters) [6]. Globally, genotype 1 predominates, accounting for 44% of all HCV infections, followed by genotype 3 (25%) and genotype 4 (15%). Genotype 1 is most common in high-income and upper-middle income countries, while genotype 3 predominates in lower middle-income countries and genotype 4 in low-income countries [7].

Natural history

Most persons do not exhibit any symptoms after acquiring HCV and as a result therefore are unaware of being infected. When symptoms do occur in the acute phase after infection, they are nonspecific and can include: fever, fatigue, abdominal pain, loss of appetite and occasionally jaundice [8, 9]. In around 25% of persons HCV is spontaneously cleared without treatment, and clearance rates may be up to 45% in children, young women and persons who developed jaundice in the acute phase

[10, 11] (Figure 1). Among MSM with HIV, spontaneous viral clearance is estimated to occur in only 11 to 18% [12-15]. Factors that are negatively associated with spontaneous clearance include male sex, older age, black race, asymptomatic HCV infection, non-genotype 1 HCV infection, and the absence of hepatitis B virus co-infection [16-18]. A strong host immune response against HCV supports clearance, and variation in genes involved in the immune response contribute to the ability to clear the virus [19, 20]. Interferon lambda 3 (IFNL3), previously known as IL-28B, is a cytokine of the type III interferon family that plays an important role in the protection against viral infections [21]. Genetic variation in the IFNL3 gene is associated with spontaneous clearance of HCV and individuals with the CC genotype are more likely to clear HCV than individuals with the CT and TT genotypes [22]. HCV infection is usually cleared within 6 months, and only few cases clear the virus 12 months after infection [11, 18].

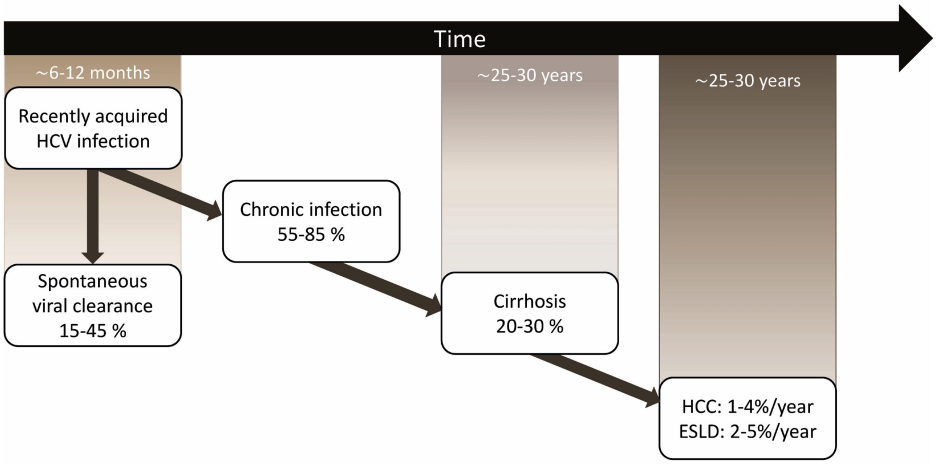

Figure 1. Natural history of HCV infection
Following infection with HCV, around 15-45% of persons spontaneously clear the virus in the first 6 to 12 months. About 55-85% with recently acquired HCV infection transition to chronic HCV infection. Among those with chronic infection, around 20-30% develop cirrhosis during a 25- to 30-year period after HCV acquisition. Persons with HCV-related cirrhosis have a 1-4% risk per year to develop hepatocellular carcinoma (HCC) and a 2-5% risk per year to develop end-stage liver disease (ESLD). Figure based on Lingala et al. 2015 [10]

Approximately 55 to 85% of persons develop a chronic HCV infection, which has an unpredictable course. Among persons with chronic HCV infection, if left untreated an estimated 20 to 30% will develop liver cirrhosis over a 25 to 30-year period. Individuals who develop HCV related cirrhosis have a 1 to 4% annual risk to develop hepatocellular carcinoma (HCC) and a 2 to 5% annual risk of progressing to end-stage liver disease (ESLD)[10, 23]. Longer HCV infection duration, male gender and older age at time of HCV infection are major risk factors for severe liver fibrosis,

cirrhosis and HCC [24]. Serious liver complications, caused by HCV, were responsible for 290,000 deaths in 2019 globally [25].

Diagnosis of HCV infection and reinfection

The first HCV diagnostic test was developed in 1989, shortly after the discovery of the virus in the same year [26]. The test was an immunoassay detecting circulating HCV antibodies. Subsequently, additional HCV antibody assays were developed with improved sensitivity. This was followed by the development of polymerase chain reaction (PCR) based HCV RNA assays, and later on, a first HCV core antigen (HCVcAg) test was developed. Both HCV RNA and HCVcAg tests measure the presence of HCV in blood (i.e. viraemia).

The traditional diagnostic algorithm uses a two-step approach: an HCV antibody test to determine exposure followed by a test to confirm viraemia, usually an HCV RNA test. For this approach trained staff, venipuncture and visits to a healthcare facility are needed. There are simplified test approaches for HCV diagnosis to improve access, including the use of dried blood spot (DBS) tests, point-of-care tests, rapid tests and HCVcAg tests [27]. The advantages and disadvantages of the currently available tests are highlighted in Table 1. Furthermore, the type of sample needed, window period (i.e. the time between HCV exposure and when HCV can be detected by the test) and average cost of each test are given.

HCV antibodies become detectable on average 28-70 days after exposure and 97% of infected persons test positive after 6 months [28]. Among persons who inject drugs (PWID) who do not have HIV, HCV antibodies can be measured within 30-60 days after exposure [29, 30]. In MSM living with HIV, HCV antibody responses may be delayed or absent, with a median time of 70-90 days from HCV exposure to seroconversion [31, 32]. This delay in seroconversion makes the HCV antibody test not the best choice for early diagnosis of HCV in MSM with HIV. Another disadvantage of the HCV antibody test is that it cannot differentiate between a recently acquired, chronic, or resolved (i.e. cleared or successfully treated) infection. A positive HCV antibody test result indicates a current viraemic HCV infection or a past HCV infection that has resolved. Since antibodies remain positive in the majority of individuals following clearance of the primary infection [31], serologic tests cannot be used to diagnose an HCV reinfection. A positive HCV antibody test needs to be followed by a test for HCV RNA or HCV core antigen (HCVcAg) to identify a viraemic infection.

HCV RNA is the most sensitive and earliest marker of HCV infection, which can be detected in blood as early as 1 week after exposure to the virus [33]. Detection of HCV RNA in the blood by nucleic acid testing (NAT) has become the gold standard for the diagnosis of HCV infection [34].

HCV core antigen is a HCV-specific marker that correlates well with HCV RNA levels and can be detected 12-15 days after exposure [35-37]. The detection of HCVcAg in the blood also confirms viraemic infection. HCVcAg tests have been developed with good performance characteristics. For the diagnosis of chronic HCV the HCVcAg test has a sensitivity of 98.8% and specificity of 99.0% [38]. For the diagnosis of recently acquired infection HCVcAg has a lower sensitivity of 87-89% [39, 40]. The slightly reduced sensitivity, compared to HCV RNA, is considered to be a disadvantage of the HCVcAg test. The advantage of the HCVcAg test is that it is a less expensive option than HCV RNA testing. Although not yet widely available in many settings, the HCVcAg may be considered as an alternative cost-saving test, depending on the laboratory set up, to use for more frequent testing in populations at high risk of HCV infection. In this thesis, the use of the HCVcAg test for the diagnosis or recently acquired HCV infection during routine care of MSM at risk of HCV infection is explored (**chapter 3**).

Alanine aminotransferase (ALT) is an enzyme found mostly in liver cells. Apart from the liver, it is also found in kidney, heart and muscle cells. The ALT test measures the level of ALT in the blood and is generally low in healthy individuals. The majority but not all individuals with acute and chronic liver disease have elevated ALT levels, which indicate liver damage [41].

ALT is not a diagnostic HCV test but it can be used as a non-specific HCV screening method. Elevation of ALT is common following initial HCV infection by about 6 to 12 weeks. In a retrospective study, Thomson et al. showed that in MSM with HIV, 88% experienced elevated ALT levels within 3 months of HCV infection [32]. A Dutch study of Vanhommerig et al. showed that in MSM living with HIV, 72% of individuals with a primary infection had elevated ALT levels at the date of first RNA-positive test, but in those with a reinfection only 44% experienced elevated ALT at the date of the first RNA-positive test [31]. This low sensitivity of the ALT test does not allow its use as a screening tool for HCV reinfection, as more than half the reinfections will be missed. As ALT levels can remain normal or rapidly normalize after infection, this testing strategy will not capture all infections if testing frequency is not sufficient [42, 43]. Furthermore, ALT may be elevated as a result of various other reasons, including drug-induced hepatotoxicity, other viral infections affecting the liver, and alcohol use [44].

Table 1. Advantages and disadvantages of different HCV diagnostic tests

Test Type	Window period	Sample type	Test cost	Advantage	Disadvantage
Antibody test	28-70 days [28]	• Venous blood • Capillary blood • Oral fluid	€	• High sensitivity & specificity [45-50] • Low cost • PoC (oral fluid and capillary blood) and DBS antibody tests available • Easy sampling options for PoC and DBS tests (finger prick, oral swap) • PoC test gives same day result (in 20-40 minutes) • PoC test can be used as self-test • PoC test and DBS sampling can be used in outreach settings • DBS can be used for self-sampling • Dried DBS sample can be easily transported • Antibodies remain stable on DBS card	• Window period relatively long • Longer window period for immunocompromised individuals (e.g. MSM with HIV) (average 70-90 days) [31, 42] • Cannot differentiate whether HCV infection is recently acquired, chronic, or no longer present • Positive test needs to be followed by HCV RNA or HCVcAg test to confirm viraemic infection

Table 1. Continued

Test Type	Window period	Sample type	Test cost	Advantage	Disadvantage
HCV RNA test	7-14 days [33, 51]	• Venous blood • Capillary blood	€€€	• High sensitivity & specificity (=gold standard) • Short window period • Indicates viraemic infection • Can determine whether person with positive HCV antibody test has an viraemic or resolved HCV infection • PoC and DBS RNA tests available • Easy sampling option for PoC and DBS tests (finger prick) • PoC gives same same day result (in 60-120 minutes) • DBS sampling can be used in outreach settings • DBS can be used for self-sampling • Dried DBS sample can be easily transported • RNA remains stable on DBS card	• High cost • PoC device needed for PoC RNA test and trained professional to operate device • PoC test does not give immediate result: waiting time up to 90 minutes • PoC test has lower sensitivity than RNA test on plasma/ serum • DBS test has lower sensitivity than RNA test on plasma/ serum [52]

Table 1. Continued

Test Type	Window period	Sample type	Test cost	Advantage	Disadvantage
HCVcAg test	12-15 days [37]	• Venous blood • Capillary blood	€€	• Short window period • Lower cost than RNA • Can determine whether person with positive HCV antibody test has an viraemic or resolved HCV infection • DBS HCVcAg test available • Easy sampling option for DBS test (finger prick) • DBS sampling can be used in outreach settings • DBS can be used for self-sampling • Dried DBS sample can be easily transported by regular mail • HCVcAg remains stable on DBS card	• Lower sensitivity than RNA test on plasma/serum [53] • Lower sensitivity for diagnosis of recently acquired than of chronic infections [39, 40] • DBS HCVcAg test has lower sensitivity than HCVcAg test on plasma/serum [50, 54, 55] • System that can run HCVcAg test is needed

Abbreviations: MSM: men who have sex with men; HIV: human immunodeficiency virus; PoC: point of care; HCV: hepatitis C virus; DBS: dried blood spot.

Point-of-care (PoC) tests are tests that are performed close or near where the individual is receiving care. PoC tests give a fast result, reduce the number of healthcare visits and may increase the likelihood of patient engagement and retention in care. POC tests have been developed to measure HCV antibodies and HCV RNA, and have made it possible to provide same-day results, with minimally invasive sampling methods. The OraQuick HCV rapid antibody test can be used with venous and capillary blood and oral fluid samples and gives a test result in 20 to 40 minutes. The test has good diagnostic performance with 99.7% sensitivity and 99.9% specificity for venous and finger prick blood. Sensitivity for oral fluid is slightly lower at 98.1% and similar specificity at 99.6% [56].

PoC HCV RNA assays also demonstrate good diagnostic performance in various settings and populations. The tests require blood samples obtained by venipuncture or finger prick and they have a turnaround time of 60 to 120 minutes. A recent systematic review and meta-analysis, evaluating five different HCV RNA assays, found a pooled sensitivity of 99% and specificity of 99% [57]. Sensitivity for finger prick is slightly lower compared to serum or plasma samples: 98% versus 100% [57]. Another systematic review and meta-analysis demonstrated that turnaround times between HCV antibody test and treatment initiation was shorter with PoC HCV RNA tests (median of 19 days [95% CI 14-53]) than with laboratory-based standard-of-care HCV RNA tests (median of 67 days [95% CI 50-67]). Furthermore, treatment uptake was higher with PoC HCV RNA tests (77%) than with laboratory-based HCV RNA tests (53%)[58]. This reduction in time for a confirmed diagnosis and the immediate engagement in care is a great advantage of PoC testing.

PoC tests can be offered in community or outreach settings, as venipuncture is not needed and the test can be performed by a non-specialist. By shifting testing from the traditional healthcare environments to community settings, various identified barriers to testing may be overcome such as lack of time, lack of transportation to a testing location, stigma, lack of access to healthcare provider and the need for multiple visits to a healthcare facility [59-61].

Dried blood spot (DBS) sampling is an alternative method to collect specimens for testing which does not require venipuncture. To sample a DBS, drops of finger prick blood are spotted on filter paper which are subsequently dried at room temperature (Figure 2). Usually 5 bloodspots are sampled but more can be needed depending on the tests that will be performed. DBS can be easily transported using regular mail to a laboratory for testing. DBS samples are easy to collect, painless, low cost, and can be performed by a non-specialist outside a healthhighcare environment. HCV antibodies, HCV RNA and HCVcAg can be measured in DBS. A systematic review and meta-analysis, evaluating the diagnostic accuracy of measuring these three HCV-specific markers using DBS, estimated the sensitivity of HCV antibody test

in DBS at 95% and specificity at 99% compared with venous blood samples. The sensitivity and specificity of HCV RNA tests in DBS compared with venous blood samples was estimated at 95% and 97% respectively. For the detection of HCVcAg in DBS the sensitivity was 86% and specificity was 98% compared with HCV RNA [55]. DBS samples may facilitate diagnosis of HCV by reflex testing on the same sample; one spot is tested for HCV antibodies, and if positive, the next spot is tested for HCV RNA [50].

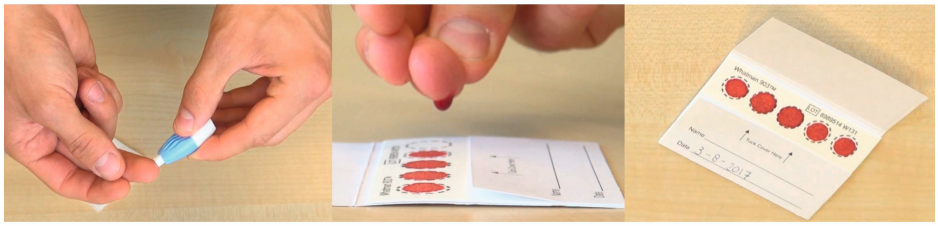

Figure 2. Sampling of a dried blood spot (DBS)
After washing the hands and disinfecting the fingertip, a finger prick is made with a lancet (left photo). To fill the circles, 1-2 droplets of blood are dropped onto each circle on the card (middle photo). The blood spot card is left to dry overnight (right photo).

As DBS samples are easy to collect, they may also be self-sampled at home for HCV testing. In this thesis the feasibility of self-sampling at home for HCV RNA detection is discussed (**chapter 2**). Self-sampling may lower barriers to HCV testing, especially if the testing service is offered anonymously. This novel testing strategy of using a home-based self-sampled DBS HCV RNA testing is described and evaluated in this thesis (**chapter 4** and **chapter 5**).

Treatment
Treatment of HCV is directed at achieving a sustained virological response (SVR), defined as undetectable HCV RNA in blood following completion of antiviral therapy. When SVR is achieved, the individual has been cured of HCV. Curing HCV has benefits for the both individual as well as for public health. At an individual level, a cured HCV prevents the progression of liver fibrosis and subsequently reduces the chance of developing HCV-related liver diseases. [62]. Being cured of HCV might subsequently improve quality of life and lift the stigma a person with HCV may experience [63]. At a public health level, curing HCV prevents onward transmission of HCV to others in the community (treatment as prevention (TasP)).

Since the discovery of HCV, more than 30 years ago, major advances have been made in treatment for HCV. The introduction of highly effective, all-oral, short duration direct-acting antiviral (DAA) treatment of HCV has by far been the most important change in the HCV landscape in recent years.

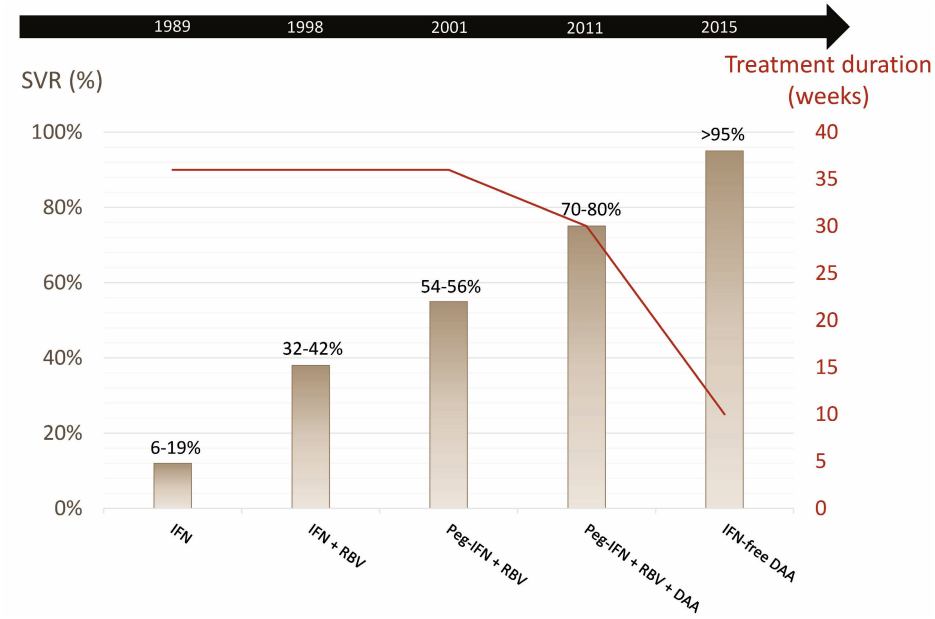

Figure 3. Development of HCV therapies over time
SVR rates for patients with HCV according to the treatment regimens and average treatment duration of treatment are shown. Abbreviations: SVR: sustained virological response; IFN: interferon; RBV: ribavirin; Peg-IFN: pegylated interferon; DAA: direct-acting antiviral agent

In the late 80's hepatitis C treatments with interferon alpha (IFN) injections for 24 weeks had a disappointing cure rate of 6% [64] (Figure 3). Extending the IFN treatment length to 48 weeks raised SVR rates to approximately 19%. Since 1998, HCV was treated with a combination therapy of interferon and oral ribavirin, improving the cure rate to 34%-42% [65]. From 2001, virological response further improved with the introduction of pegylated interferon (Peg-IFN) combined with ribavirin, resulting in cure rates of 45% for genotype 1 and 70-80% for genotype 2 and 3 [66, 67]. Treatment with Peg-IFN and ribavirin was often accompanied by serious side effects such as anemia and depression [68]. In 2011, a major breakthrough occurred in HCV treatment with the development of a therapy directed at the virus itself. Protease inhibitors were the first direct-acting antivirals (DAAs) to show promise: the addition of protease inhibitors to Peg-IFN-ribavirin therapy led to cure rates of 70-80% [69, 70]. Subsequently, the treatment of HCV was revolutionized by the development of more potent DAAs. These DAA regimens are IFN-free, provide excellent cure rates of over 95%, have hardly any side effects and short treatment duration of 8 to 12 weeks [71, 72]. DAAs were initially genotype-specific, but currently pan-genotypic regimens are available.

Even though effective treatments for HCV now exist, diagnosis and linkage to care remains a bottleneck in many countries: of the estimated 12.9 million people who had been diagnosed in 2020 around 641,000 (~5%) patients started treatment in that year [73]. In the Netherlands, the first DAA regimens became available for patients with chronic HCV in 2014, but were only reimbursed for patients with severe liver fibrosis. In November 2015, the reimbursement criteria were expanded to give unrestricted access to DAA treatment for all chronic HCV patients, regardless of liver fibrosis stage. Fifteen months after DAAs were made available, 76% of the HIV/HCV-coinfected patients in care were successfully treated for HCV and another 6% were still undergoing treatment or awaiting treatment results. Treatment uptake and HCV cure rates were highest in MSM; 83% had their infection cured and in 6% DAA results were pending [74].

Is has been commonly accepted that treating recently acquired HCV is as successful as treating chronic HCV [75]. While DAAs are not yet registered for treatment of recently acquired HCV in the Netherlands, substantial delays in treatment initiation are not common when no spontaneous clearance is observed. When there is no decrease in HCV RNA of >2Log10, 4 weeks after diagnosis, prompt DAA treatment is recommended [76].

HCV epidemiology

At the beginning of 2020, an estimated 56.8 million people were living with HCV worldwide [73]. The World Health Organization (WHO) estimated that globally 1.5 million new infections occur every year. Only 21% of people living with HCV is diagnosed and of those who require treatment a small fraction (~5-13%) has access to it [25, 73]. In 2019, 290,000 persons died due to complications of HCV, mostly cirrhosis and hepatocellular carcinoma [77]. In the recently published global progress report on HIV, viral hepatitis and sexually transmitted infections, WHO stresses that the current high HCV burden and associated mortality are unacceptable, and describes the urgent need to expand HCV diagnosis and treatment services to reach most affected subpopulations [25]. Early detection and treatment are crucial to reduce the global burden of HCV and associated mortality.

There are significant differences in HCV prevalence and incidence globally (Figure 4), with some regions and subpopulations being disproportionally affected. In high-income countries HCV prevalence (defined as the percentage of the total population with a viraemic infection (HCV RNA positive)) is typically lower due to better access to healthcare and resources for disease prevention and treatment. In the group of high income countries (as defined by the World Bank) the HCV prevalence was 0.5% in 2020 [73]. However, certain populations, such as PWIDs and people living with

HIV, MSM, people with inherited blood disorders, and prisoners still have a higher HCV prevalence [25, 78-80]. In low- and middle-income countries, the burden of HCV is much higher, particularly in regions with high rates of poverty, poor access to healthcare, and limited resources for disease prevention and treatment. For example, in Eastern Europe and Central Asia the prevalence in 2020 was 2.9% and 2.6% respectively, and the largest number of viraemic infections were estimated in south Asia (14.5 million) and east Asia (10.0 million) [73].

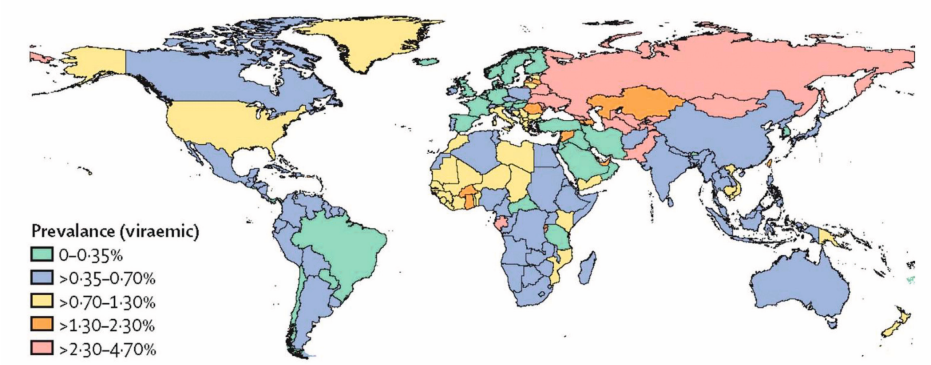

Figure 4. HCV prevalence 2020
The viraemic HCV prevalence for all countries and territories, including those with an extrapolated prevalence in 2020. Figure from Polaris Observatory HCV Collaborators, Lancet Gastroenterology & Hepatology [73].

Globally, the burden of HCV is highest among PWID, with an estimated prevalence greater than 40% [79, 81]. This high prevalence among PWID reflects the significant risk of HCV transmission through sharing injecting equipment and the need for comprehensive harm reduction strategies, including needle exchange programs and opioid substitution therapy [82]. Promoting decriminalization of drug use and expanding HCV treatment services among PWID may also contribute to HCV prevention in this population [83].

As both HIV and HCV share the same transmission routes, people with HIV are a population at risk for HCV. Additionally, coinfection with HIV reduces the HCV spontaneous clearance rate and accelerates liver disease progression in untreated individuals [84]. Among MSM, HCV prevalence is also higher, with the main risk factors for HCV acquisition being drug use and certain sexual practices [85] (epidemiology, risk factors and prevention strategies for this key population are described later in this chapter). The population of incarcerated people also have a high burden of HCV, because of a combination of factors including injection drug use, risky sexual practices, tattooing, body piercing, and sharing of personal care items [83, 86]. Prevention programs to reduce HCV in prisons, including harm reduction and HCV testing and treatment services should be offered.

Unsafe medical procedures are another significant factor contributing to the epidemic. Before the introduction of blood donor screening in the mid to late 1990s, blood transfusion and infected healthcare injections were the leading causes of HCV transmission. However, other sources of iatrogenic transmission have been described, including organ transplantation from donors with HCV, dental care and surgery [87]. Prior to the widespread screening of blood supply, nearly all patients with inherited blood disorders (e.g. hemophilia) acquired HCV by contaminated blood transfusion [88]. Many people who need hemodialysis have had multiple potentially unscreened blood transfusions in the past, and nosocomial infections continue to occur in dialysis units globally, making people who need hemodialysis a population at HCV risk. Significant progress has been made to reduce the transmission in medical settings, but is uneven across regions. Most high-income countries have safe healthcare practices in place, but in low- and middle-income countries, unsafe healthcare injections still occur and blood screen coverage is insufficient [89]. Prevention of HCV in low-and middle-income countries should be part of a more comprehensive approach to strengthen health systems and to improve safety, including infection control, provision of clean needles and screening of blood donations.

In 2016, the global burden of HCV together with the introduction of highly effective DAAs prompted the WHO to release its first Global Health Sector Strategy on Viral Hepatitis [90]. The strategy called for elimination of viral hepatitis B and C infection as a public health threat and ambitious targets were set. To reach HCV elimination, countries worldwide are to reduce HCV incidence by 80% and HCV-related deaths by 80% by 2030, compared with the 2015 baseline [91]. In 2021, WHO proposed thresholds of ≤ 5 new HCV infections per 100,000 person-years and ≤ 2 HCV-related deaths per 100,000 person-years, as evidence of HCV elimination [92]. This is an enormous public challenge and stakeholders may experience working towards nation-wide elimination as overwhelming and complex. Therefore, a micro-elimination approach has been recommended.

Micro-elimination

Micro-elimination refers to the targeted elimination of a specific pathogen in specific populations and/or geographic areas, with the aim of reducing the overall burden of disease. In 2017, micro-elimination of HCV was proposed by the European Association for the Study of the Liver's (EASL) International Liver Foundation, as a pragmatic approach to nation-wide HCV elimination by "breaking down national elimination goals into smaller goals for individual population segments, for which treatment and prevention interventions can be delivered more quickly and efficiently using targeted methods" [93]. The HCV micro-elimination approach typically focusses on populations with high HCV incidence (described above), and encourages stakeholders who are familiar with specific populations to collaborate. Tailoring prevention interventions and making HCV testing and treatment services more accessible to the defined populations are part of a successful micro-elimination strategy, and should be implemented through a multi-stakeholder process [94]. This collaborative approach among relevant parties may facilitate the implementation of tailored interventions and new models of care and effectively reach target populations. An example of such a new model of care is the co-location of services (e.g. testing and treatment services within the same building or location) or providing HCV testing and treatment sites outside the healthcare environment [95-97].

In this thesis, the multi-stakeholder development process and implementation of a micro-elimination strategy to stop the transmission of HCV among MSM in Amsterdam is described (**chapter 4**). A modelling study by Martin et al. indicated that HCV treatment scale-up alone is not likely to reach the WHO target of 80% reduction in new HCV infections among MSM with HIV in Berlin from 2015 to 2030 [98]. However, combing DAA scale-up with 40% behavioral risk reduction among MSM with HIV and 20% reduction of background incidence from outside the group of MSM with HIV (e.g. MSM without HIV and PWID) in 2018, a 81.2% reduction in HCV incidence could be reached, from 2015 to 2030 [98]. Other modelling studies have also indicated that early treatment in combination with the implementation of health promotion interventions that reduce high-risk behavior is an effective strategy to control the HCV epidemic in MSM [99, 100]. Therefore, we aimed to develop a comprehensive strategy that aimed to increase HCV knowledge and awareness, promote regular testing, provide fast linkage to care, promote risk reduction behavior, and enhance partner notification among MSM.

HCV among men who have sex with men

The studies in this thesis are focused on one key population: MSM in the Netherlands, and in some chapters on MSM in Amsterdam. In 2005, a report was published describing the sexual transmission of HCV among MSM living with HIV in Rotterdam, the Netherlands [101]. Subsequently, a retrospective study was published in 2007 describing an increase in HCV incidence from 1984 to 2003, among MSM with HIV in Amsterdam [102]. Furthermore, phylogenetic analysis of the cohort of MSM with HIV/HCV co-infection supported the presence of an MSM-specific transmission network in Amsterdam, suggesting sexual transmission [102]. Among MSM living with HIV attending the sexual health clinic in Amsterdam a high and increasing HCV prevalence was found from 2007 to 2008 [103]. The transmission of HCV among MSM living with HIV was not unique to the Netherlands as HCV outbreaks were reported globally since 2000 [104, 105]. Reports from Australia, the USA, Germany, France, Switzerland, and the UK demonstrated the presence of a worldwide epidemic among MSM living with HIV in urban settings [105-110]. Phylogenetic analysis revealed the existence of a large international MSM-specific transmission network that linked the national HCV outbreaks [105].

In the Netherlands, a decline in primary HCV infection and HCV reinfection incidence was observed in MSM living with HIV, in HIV care, after the introduction of unrestricted access to DAAs in 2015 [111]. Between 2008 and 2015 the HCV incidence fluctuated between 8.7 and 13.0 per 1000 person-years and significantly declined to 6.1 per 1000 person-years in 2016. Between 2017 and 2019, HCV incidence fluctuated between 4.1 and 4.9 per 1000 person-years. HCV reinfection incidence also declined from 41.4 per 1000 person-years in 2016 to 11.4 per 1000 person-years in 2019, but it remained relatively high, illustrating ongoing HCV transmission in MSM living with HIV in the Netherlands [111]. This downward trend in HCV incidence among people with HIV has also been observed in other high income countries from 2010 to 2019, which suggests that broad DAA access has a treatment as prevention effect on primary HCV incidence [112].

Insight into HCV incidence among MSM without HIV is more challenging as they are normally not in routine clinical care. Among this group, several studies reported small numbers of incident HCV infections [113-115]. In the period 2007-2010 the HCV prevalence among MSM without HIV, attending the center of sexual health in Amsterdam was low (0.5%) and the majority of HCV strains found in this population were neither closely related to strains circulating among MSM living with HIV nor were they closely related to each other [113]. This demonstrated that MSM without HIV acquired HCV through unrelated transmission events. However, in this study an MSM-specific strain was reported among one of the MSM without HIV, which suggested there was some overlap with the sexual transmission networks affecting

MSM with HIV [113]. In the Amsterdam Cohort Study, no incident HCV infection was found among MSM without HIV in the period from 1984-2011, despite more than 10,000 person-years follow-up [114]. Two systematic reviews showed a 16-to-19-fold lower HCV incidence in MSM without HIV compared to MSM with HIV, including studies from 2000 to 2016 (0.4 per 1000 person-years in MSM without HIV and 6.4–7.8 per 1000 person-years in MSM with HIV) [116, 117]. Explanations have been suggested why MSM without HIV remained largely unaffected by HCV, being: their lower biological susceptibility to HCV compared to MSM with HIV, serosorting (having sex with partners of the same HIV-status) and lower sexual transmissibility of HCV compared to HIV, explaining why HCV is often preceded by an HIV infection [85, 118, 119]

The introduction and increased uptake of prescribed pre-exposure prophylaxis (PrEP) to prevent HIV infection, has resulted in changes in sexual behavior among MSM without HIV and increasing overlap in sexual networks between MSM living with and without HIV [120, 121]. A meta-analysis, including studies from 2000 to 2019, estimated a 123-fold higher HCV incidence in MSM without HIV using PrEP compared to MSM without HIV not using PrEP and a pooled HCV incidence of 14.8 per 1000 person-years in PrEP-using MSM [122]. Similarities and differences in HCV incidence among PrEP-using MSM have been observed between countries, with high incidence rates in the Netherlands, UK and France, and low incidence rates in Canada and Australia. The AmPrEP study in Amsterdam reported an HCV incidence of 23 per 1000 person-years among participants [121]. The PROUD study in London reported an HCV incidence of 19 per 1000 person-years [123]. The French ANRS IPERGAY study and a French hospital-based cohort reported similar HCV incidence of 14 per 1000 person-years [124, 125]. A lower incidence rate of 7 per 1000 person-years was found among participants using daily PrEP in the ANRS Prevenir study in Paris. In contrast, in the BC PrEP program in Canada and the PrEPX study in Australia, low rates of 2 and 4 per 1000 person-years were reported respectively [126, 127]. In Amsterdam, the high HCV reinfection incidence among PrEP-using MSM is of additional concern. During follow-up in the AmPrEP study from 2015 to 2018, an HCV reinfection incidence of 278 per 1000 person-years was found [121]. These high incidence rates were found in groups of early adopters of PrEP. HCV primary and reinfection incidence may be different among the larger population of PrEP users, who now have access to PrEP.

Transmission, risk factors and prevention

HCV transmission among MSM occurs mainly through sexual contact [85, 128]. Several studies have identified certain sexual techniques and settings as risk factors for primary HCV infection, including condomless anal intercourse, (unprotected) fisting, sharing of sex toys, group sex and sexualized drug use [85, 128]. In addition, having an ulcerative sexually transmitted infection (STI), injecting drug use, sharing

of straws or other equipment for snorting drugs, sharing anal douching equipment and rectal bleeding were also found to be associated with an increased risk of HCV infection [85, 128]. A recent study in the Netherlands found that receptive condomless anal intercourse, sharing of sex toys, group sex, anal rinsing before sex, having 10 or more sex partners in the last 6 months were also associated with HCV reinfection [129].

At present, a vaccine to prevent HCV is unavailable and as HCV treatment scale-up alone is not likely to reach a substantial reduction in new HCV infections, the prevention of (re)infections among MSM should include scaling up HCV treatment in combination with behavioral risk reduction interventions for MSM [98, 99]. Interventions aimed at reducing sexual risk in MSM have primarily focused on preventing HIV transmission through condom use, and more recently PrEP use. However, for MSM who are at risk of HCV (re)infection, new and expanded strategies are needed to target sexual behaviors that are specifically associated with HCV transmission. Informing men at risk about the possible HCV risk factors other than anal intercourse, as well as motivating them to integrate related risk reduction strategies into their sex lives and promote regular HCV testing are essential. The first HCV specific sexual risk reduction intervention was developed in 2016 in Switzerland as part of the HCVree trial [130]: the intervention for HIV/HCV co-infected MSM provided DAA treatment in combination with individual counselling sessions aimed at reducing sexual risk taking [131]. A qualitative evaluation of the intervention showed a diversity of responses from men who had received DAA treatment and counseling [131]. Some actively modified their risk behavior and felt supported by the intervention to maintain their changes in behavior. Some minimized risk by adopting behavior changes that suited them personally. Others perceived behavior change as much more difficult than the “easy” DAA treatment option and accepted living with the risk of HCV reinfection [131]. Two years after the HCVree trial, the long term impact of the intervention on the HCV prevalence and incidence among MSM was assessed, and showed a sustained effect: prevalence of replicating HCV infection reduced from 1.2% to 0.6% and HCV incidence reduced from 3 to 2 per 1000 person-years [132].

Co-creation

Using a top-down approach and one size fits all interventions have had limited success in health behavior change, especially in contexts where a complex set of factors influence behavior across individuals and settings [133]. Tailoring interventions, by matching strategies to individual or group needs in specific settings, has the potential to address complex health issues more effectively [134]. End-users and other non-academic stakeholders (such as peers) should work together with

academic stakeholders to co-create interventions to guarantee that interventions are properly tailored to the target end-user and settings [135]. Involving end-users in the design and development of products and services is not new; in marketing [136], economics [137] and business [138] there is extensive experience in making products more appealing, marketable and to increase customer loyalty by end-user engagement [139]. The degree of engagement varies from little input to full control of the product or service development process. Examples of the involvement are: getting end-user feedback on a designed product or service, involving all stakeholders throughout the development process and the starting and controlling a design by end-users [140]. The involvement of end-users in the co-creation of public health interventions is believed to increase both the intervention effectiveness and the extent to which health behavior recommendations are followed [141]. Co-creation results in contextually appropriate intervention strategies and creates a platform for co-learning and enhances ownership and empowerment among the target group [142, 143].

The development of the multilevel intervention aimed at reducing HCV transmission among MSM in Amsterdam, discussed in this thesis, used a co-creation approach and actively involved healthcare professionals, researchers and the target group, MSM at risk of HCV. The co-creation process is described in detail in **chapter 4**. To the best of our knowledge, the involvement of the target group in the development of an HCV prevention intervention has not previously been reported, making this intervention unique and innovative. The approach taken ensured the support of the various intervention components including socio-culturally and contextually appropriate information about HCV and risk reduction.

Information Motivation Behavioral skills model

The multilevel intervention (described in **chapter 4**), included both web-based and face-to-face components as well as an anonymous HCV testing service. A website providing information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options, and partner notification, was developed. Furthermore, a risk reduction toolbox, training for health professionals, and tailored advice to sex on premises venues was provided. The development of the risk reduction toolbox (described in **chapter 6**), was guided by the Information Motivation Behavioral skills (IMB) model [144]. The IMB model was chosen as our theoretical framework as it has been shown to be effective in preventing risky sexual behaviors among different key populations [145-147]. Three primary constructs that influence behavior changes are included in the IMB model: information and knowledge about behavior, the individual's motivation to perform the behavior, and the behavioral skills necessary to perform the behavior [144]. The IMB

model suggests that information and motivation predominantly work through the behavioral skills component to influence behavior (Figure 5). Information and motivation can also influence behavior directly, especially for behaviors that require minimal skill. The IMB model recognizes that information and motivation may be intercorrelated.

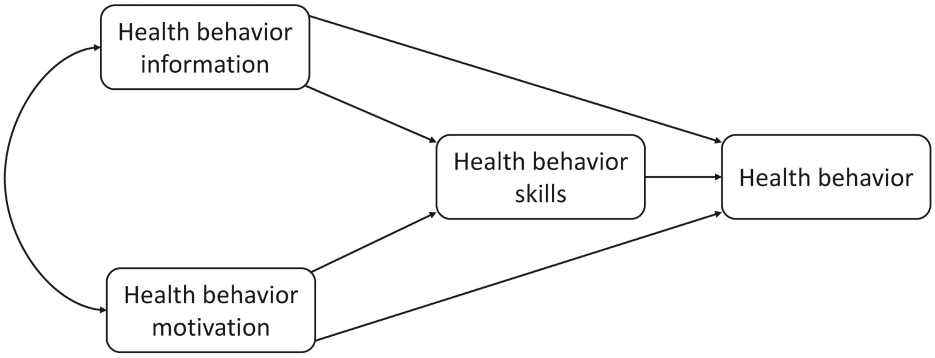

Figure 5. The information Motivation Behavioral Skills model
Figure based on Fisher and Fisher 1992 [144]

A substantial amount of research has demonstrated the usefulness of the IMB model as a framework for public health prevention and education [148]. The majority of this research has been on HIV prevention programs, but the utility of the IMB model has also been confirmed in a variety of other fields such as programs to improve long-term adherence to chronic hepatitis B infection monitoring [149], improve adherence to antiretroviral therapy [150], and osteoporosis prevention behavior [151]. It has been proposed that the IMB model provides a general working framework, which can be applied to any health education of prevention program development and assessment [152, 153]. The simplicity and robustness of the IMB model provided a useful framework for the development and evaluation of the intervention component aimed at promoting HCV risk reduction behavior in MSM at HCV risk. The IMB model applied to the HCV context, proposes that individuals will initiate and maintain HCV preventive behaviors if they are well-informed regarding HCV, HCV transmission routes, HCV infection risks, and possible preventive behaviors, are motivated to prevent infection, possess the skills and perceive themselves as being capable of applying the recommended preventive strategies. **Chapter 6** gives a detailed description of how the IMB model constructs have been integrated in our intervention.

Aims and outline of thesis

The overall aim of this thesis was to describe and evaluate innovative approaches to testing and prevention of HCV among MSM regardless of their HIV status, in Amsterdam, which may contribute to HCV micro-elimination in this population.

The first two chapters of this thesis focuses on additional and alternative options for HCV testing and diagnosis. In **chapter 2**, the feasibility of self-sampling dried blood spots at home was evaluated, for HCV RNA detection. In **chapter 3**, the use of HCV core antigen was evaluated for the diagnosis of recently acquired HCV infections among MSM in the Netherlands.

The subsequent chapters focus on the development, uptake and evaluation of a multilevel intervention to reduce HCV transmission among MSM in Amsterdam. In **chapter 4** the implementation and development of this intervention through a co-creation process were described. **Chapter 5** focusses on one of the intervention components, the HCV RNA home-based self-sampling test service. The use and outcomes of the test service were evaluated and user experiences reported. In **chapter 6**, the development of another intervention component, the risk reduction toolbox was described and its use and impact on behavior was assessed.

The last chapter, **chapter 7**, summarizes the outcomes of our studies, relates these to recent literature and reflects on the implementation of the multilevel intervention. It discusses the implications of our findings and gives recommendations for further research.

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Chapter 2

Dried blood spot self-sampling at home is a feasible technique for hepatitis C RNA detection

Tamara Prinsenberg, Sjoerd Rebers, Anders Boyd, Freke Zuure, Maria Prins, Marc van der Valk, Janke Schinkel

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Abstract

To facilitate HCV diagnosis, we developed an HCV RNA testing service, which involved home-sampled dried blood spots (DBS). The main objective of this study was to evaluate the feasibility of self-sampling at home. Furthermore, to optimise the processing of DBS samples for RNA detection, we evaluated two elution buffers: phosphate-buffered saline (PBS) and L6-buffer. 27 HCV RNA and 12 HIV-1 RNA positive patients were included. Laboratory spotted DBS (LabDBS) were made by a technician from blood samples drawn at inclusion. Patients received a DBS home-sampling kit and were requested to return their self-sampled DBS (ssDBS) by mail. We compared the RNA load of PBS and L6-eluted labDBS, and of L6-eluted ssDBS, L6-eluted labDBS and plasma. LabDBS load measurements were repeated after 7–13 and 14–21 days to evaluate RNA stability. All 39 plasma samples provided quantifiable RNA loads. In 1/39 labDBS sample, RNA could not be detected (plasma HCV load: 2.98 log10 IU/ml). L6-eluted samples gave a 0.7 log10 and 0.6 log10 higher viral load for HCV and HIV-1 respectively, compared to PBS-eluted samples. Strong correlations were found between labDBS and ssDBS HCV RNA (r = 0.833; mean difference 0.3 log10 IU/mL) and HIV-1 RNA results (r = 0.857; mean difference 0.1 log10 copies/mL). Correlations between labDBS and plasma values were high for HCV (r = 0.958) and HIV-1 (r = 0.844). RNA loads in DBS remained stable over 21 days. Our study demonstrates that self-sampling dried blood spots at home is a feasible strategy for the detection of HCV and HIV-1 RNA. This could facilitate one-step diagnostics and treatment monitoring in communities with high HCV prevalence.

Introduction

In the Netherlands, transmission of hepatitis C virus (HCV) occurs primarily among HIV-positive men who have sex with men (MSM) as HCV incidence dropped to nearly zero among people who inject drugs [1–3]. Since 2000, there has been an unexpected and substantial increase in HCV incidence among MSM [4, 5]. Most infections in this group are sexually transmitted and occur in human immunodeficiency virus-1 (HIV) infected MSM. However, recent studies have shown that HIV-negative MSM eligible for or on pre-exposure prophylaxis (PrEP) to prevent HIV acquisition are also at risk of acquiring HCV [6, 7].

In response to this epidemic, in 2016 we formed the Amsterdam MSM Hepatitis C Free consortium (MC Free) with the goal to stop HCV transmission among MSM in Amsterdam. In close cooperation with the MSM community, (commercial) stakeholders and healthcare professionals the NoMoreC project was developed. The project aims to increase the uptake of testing, improve the engagement of MSM in preventive behaviors and increase awareness among the population at risk and healthcare professionals. NoMoreC uses online and face-toface interventions, including a training package, a prevention toolbox to assist risk reduction and a website (www.NoMoreC.nl). The project website offers information, videos and personalized advice on risk reduction and testing options, including a HCV RNA home-based testing service. This service involves self-sampling of dried blood spot (DBS) samples by a finger-prick, mailing the samples to our laboratory for testing and receiving the test result online. The NoMoreC home-based testing service allows MSM to test shortly after engaging in at risk activity, thereby enabling them to assume responsibility of their own health. Additionally, the service offers the possibility to test for HCV outside the healthcare system, which may lower the barrier to testing and increase testing frequency.

Previous studies have shown that DBS, spotted in the laboratory, can be reliably used for the detection and quantification of HCV RNA, with reported detection limits varying between 2.2–3.4 log10 IU/mL [8–11]. Although the detection limits in DBS are 0.5–1.7 log10 IU/mL higher than in plasma, it is suitable for diagnosing patients with HCV infection, as most patients have viral loads >3.0 log10 IU/mL [9]. HIV-1 RNA testing on DBS has also been evaluated in several studies and was shown to have a limit of detection of 3.7 log10 copies/mL, 2 log10 higher than in plasma viral load assays [12].

As part of the NoMoreC testing service, DBS samples are transported by mail, which causes a delay in time between sampling and viral load measurements. As a consequence of unfavorable transport conditions, a decline of viral RNA on DBS over time could be an issue when stored at room temperature (RT). Reports on

the stability of viral RNA on DBS are inconsistent: some studies observed a three to ten-fold decline in viral load after 3 days and 4 weeks of storage at RT [9, 13], whereas others showed that HCV RNA levels on DBS remained stable at RT for over a year [14].

Only a limited number of studies have explicitly evaluated the use of self-sampled DBS (ssDBS) [15–20]. The majority of these studies reported the use of ssDBS for determining drug levels [16–19]. One large Japanese study showed the successful use of ssDBS sent by regular mail for HIV diagnosing HIV using an HIV antigen/antibody test [21].

To our knowledge, no studies have been performed to evaluate ssDBS for diagnosing a viral infection by measuring viral RNA. For a reliable HCV testing service, it is essential to assess if people can adequately sample a DBS at home and if HCV RNA can be detected in these samples without compromising sensitivity of RNA detection. Therefore, the main objective of the current study was to evaluate the self-sampling DBS technique for the detection of HCV RNA. First, we assessed the quality of the ssDBS samples and the patients' experience with self-sampling. Second, we determined the agreement between laboratory spotted DBS (labDBS) and ssDBS viral load measurements and the correlation between labDBS and plasma viral loads. Third, elution buffers were compared to optimize the processing of DBS samples for RNA detection. Fourth, effect of RT storage on viral RNA levels was also assessed on LabDBS samples. Anticipating on future direction ssDBS may take for viral diagnostics, we also include the detection of HIV-1 RNA in this evaluation. Finally, we studied the effectiveness of different elution buffers and explored the detection limit of HCVcAg in DBS.

Patients and methods

Patients

HCV or HIV-1 viremic patients were recruited during routine visits at the hepatology and infectious disease outpatient departments at the Amsterdam UMC, location Academic Medical Center, the Netherlands from September 2017 to April 2018. The study received ethical approval from the medical ethics committee of the Amsterdam UMC (Study nr. 2017_170), in accordance with the Helsinki declaration. After giving their informed consent participants received a DBS home sampling kit and 1 tube of EDTA whole blood was by a healthcare worker.

Sample collection and processing

ssDBS collection

Finger-prick blood samples were collected by the participants at home with a DBS home sampling kit. The kit contained: paper instructions, 2 contact-activated lancets (2.0-mm BD Microtainer), 2 band aids, a DBS card with 5 circles (Whatman Protein Saver 903 card; GE Healthcare), an alcohol wipe, a gauze wipe, a grip seal bag, a desiccant sachet and a return envelope (UN 3373). To assist the blood collection, participants had access to an online instructional video (https://www.youtube.com/watch?v=AyDXoTkliLI) in addition to the paper instructions. Participants were instructed to fill all 5 circles on the DBS card, to air dry at RT overnight, and to write the sampling date on the card before putting it in a grip seal bag with the desiccant and return it by mail.

User-friendliness of self-sampling

Participants received a short questionnaire with the home sampling kit and were asked to rate their experience with DBS self-sampling. The questionnaire included yes/no questions and 5-point Likert scale questions about (1) the use and clarity of the instructions, (2) ease of performing a finger prick and (3) ease of making a bloodspot. The questionnaire and paper instructions were pre-tested by 3 patients and adjusted according to their feedback.

ssDBS quality assessment

On receipt in the laboratory, ssDBS samples were visually assessed by a laboratory technician using the quality criteria as described by Hoeller et al. [20]: spot size (circle completely filled), thoroughly soaked (observed from the back) and one application of blood (not composed of many small spots). In addition, the number of good spots were counted and the reasons for failed spots were recorded. All spots were subsequently processed regardless of their quality.

Plasma and labDBS collection

EDTA whole blood samples were collected by venipuncture by a healthcare worker and stored at -80°C. HCV and HIV-1 RNA testing was performed on plasma within 7 days. LabDBS samples were obtained by spotting 10 circles on 2 cards per patient (60 µL of EDTA whole blood per circle onto Whatman Protein Saver 903 card; GE Healthcare) and air dried overnight at RT. LabDBS cards were stored at RT in grip seal bags with desiccant until processing of the DBS sample at <7 days (t0), after 7–13 days (t1) and after 14–21 days (t2).

DBS processing for testing

Two spots were cut out of the DBS card with a clean pair of scissors. For each DBS sample, a new clean pair of scissors was used to avoid cross-contamination. Each spot was cut into small pieces (6 pieces/spot) to be able to fit in an Eppendorf

tube and facilitate the elution process. The two cut up spots were placed into an Eppendorf tube (12 pieces/tube) and mixed with elution buffer. For the elution buffer comparison, only labDBS samples were used and eluted in both PBS (Phosphate Buffered Saline, BSA (10%) and Tween-20 (0.05%)) and L6-buffer (500 g GuSCN in 91.7 mL 0.2M EDTA [pH8.0], 10.12 mL Triton X-100, and 416.7 mL 0.1M Tris-HCl [pH6.4]) at t0. Preparation of the buffers is described elsewhere [9, 22]. For the elution of ssDBS and the elution of labDBS at t1 and t2, only the L6-buffer was used. Two spots (120 µL of whole blood) were eluted in 800 µL buffer for HCV RNA measurement or in 1200 µL buffer for HIV-1 RNA measurement. The tubes were shaken (1000 rpm) for 1–2 hours at RT and then centrifuged for 5 minutes at 14000 rpm. The supernatant was transferred into a new Eppendorf tube and stored at -80°C until viral load measurements.

HCV RNA and HIV-1 RNA measurements

The CAP/CTM assay (COBAS Ampliprep/COBAS TaqMan; Roche Diagnostics) was used for extraction, amplification and quantification of HCV and HIV-1 RNA on plasma and DBS eluates from 2 spots. Briefly, 650 µL and 1000 µL plasma specimens and were pipetted into the specimen tubes, for HCV and HIV-1 measurements respectively. After vortexing the specimens were transferred to the CAP/CTM for processing. For DBS eluates, the same quantities (650 µL for HCV, 1000 µL for HIV-1) and procedures were followed.

Statistical analysis

Simple descriptive statistics were used to analyse and report on the quality of the ssDBS samples and the user-friendliness of self-sampling.

HCV and HIV-1 RNA levels were log10 transformed. All analyses were run for HCV and HIV-1 separately. First, values obtained from labDBS and plasma were plotted and compared using Pearson's correlation. A linear regression model was then fit, using the plasma samples as the dependent variable and labDBS samples as the independent variable. For each pair of measurements, the differences between plasma and labDBS values were then plotted against their means in a Bland-Altman analysis. Assuming normal distribution of viral loads, the between-method difference and its limits of agreement (LOA) were calculated. The same was done comparing values obtained from ssDBS and labDBS.

Second, we examined the effectiveness of L6 and PBS buffer. LabDBS values measured in L6-eluates and PBS-eluates were plotted and compared using Pearson's correlation. A linear regression model was then fit, using the PBS buffer as the dependent variable and the L6 buffer as the independent variable. For each pair of measurements, the differences between values measured in PBS and L6-eluates were then plotted against their means in a Bland-Altman analysis. We then

evaluated the diagnostic sensitivity of HCV and HIV-1 RNA detection in LabDBS and ssDBS, eluted in L6 buffer.

For those labDBS samples with repeated viral load measurements, the difference between plasma and labDBS values were calculated and plotted over time. The average change in difference over time was estimated by mixed-effect linear regression accounting for patient variability at initial sample using a random intercept.

Statistical analysis was conducted using STATA (v12.1, College Station, TX) and a p-value <0.05 was considered statistically significant.

Results

A total of 39 viremic patients were included: 17 patients were HCV mono-infected, 11 were HIV-1 mono-infected and 11 were HCV/HIV co-infected. Of the co-infected patients, 10 had a detectable HCV viral load and undetectable HIV viral load (<40 copies/mL) and in 1 patient, both HCV and HIV-1 RNA were detectable.

Table 1 gives an overview of the study samples; the total number of plasma samples, DBS samples spotted in the laboratory (labDBS) and DBS samples spotted by the patient (ssDBS). The number of DBS samples eluted in PBS and L6 buffer is also shown.

ssDBS quality

In total, 34 of the 39 patients (87%) returned their self-sampled DBS, of which 33 (97%) were visually assessed according to the three quality criteria. Twelve out of 33 patients (36%) sampled 5/5 good quality spots that met all quality criteria and 19 patients (58%) sampled at least 2/5 spots meeting all criteria. The spots that did not meet all criteria were classified as failed. The main reason for a failed spot was that the circle was not completely filled (76% of failed spots).

User-friendliness

Of the 34 participants whom returned their ssDBS sample, 33 had filled out a questionnaire regarding their experience with DBS self-sampling and the clarity of instructions (Table 2).

Table 1. Overview of study samples.

Target measured	**Plasma samples n=39**			**LabDBS samples n=39** [α]				**ssDBS samples n= 34** [α]			
	HCV mono-infected	HCV/ HIV co-infected	Total		HCV mono-infected	HCV/ HIV co-infected	Total		HCV mono-infected	HCV/ HIV co-infected	Total
HCV	17	10	**27**	LabDBS made	17	10	**27** [α]	ssDBS received	15	7	**22** [α]
				Eluted in PBS	15	10	25 [α]	Eluted in PBS	3	1	4 [α]
				Eluted in L6	17	10	27 [α]	Eluted in L6	15	7	22 [α]
	HIV-1 mono-infected	HIV/ HCV co-infected	Total		HIV-1 mono-infected	HIV/ HCV co-infected	Total		HIV-1 mono-infected	HIV/ HCV co-infected	Total
HIV-1	11	1	**12**	LabDBS made	11	1	**12**	ssDBS received	11	1	**12**
Eluted in L6				Eluted in PBS	11	1	12	Eluted in PBS	3		3
				Eluted in L6	10	1	11	Eluted in L6	9	1	10

Total number of plasma samples drawn, DBS samples spotted in the laboratory (LabDBS) and DBS samples spotted by the patient (ssDBS). For the DBS samples the number of samples eluted by PBS and L6 buffer is shown. Numbers of samples of HCV and HIV-1 mono-infected and HCV/HIV co-infected patients are shown per target measured.

[α] : in 1 sample with a plasma viral load of 2.98 $\log_{10}$ IU/mL the target was not detected in DBS

Table 2. Patients' use and experience with DBS self-sampling kit. Results of 33 returned questionnaires.

Nr. of patients (%)	
Use and clarity of instructions (N=33)	
Number using paper instructions	32 (97%)
Paper instructions are:	
Helpful	32 (100%)
Very clear	15 (47%)
Clear	16 (50%)
Very unclear	1 (3%)
Number using video instructions	17 (52%)
Video instructions are:	
Helpful	15 (88%)
Not helpful	2 (12%)
Very clear	9 (53%)
Clear	8 (47%)
Experience with performing finger prick (N=33)	
Very easy	11 (33%)
Easy	9 (27%)
Not easy/not hard	6 (18%)
Hard	4 (12%)
Very hard	1 (3%)
Not answered	2 (6%)
Experience with filling circles correctly (N=33)	
Very easy	2 (6%)
Easy	14 (42 %)
Not easy/not hard	8 (24%)
Hard	6 (18%)
Very hard	2 (6%)
Not answered	1 (3%)

In total, 32 participants (97%), used the paper instructions and found these helpful. The instructions were rated as very clear by 15/32 participants, clear by 16/32 participants and very unclear by one participant. 17 participants (52%) used the instructional video in addition to the paper instructions and rated the video as clear (8/17) or very clear (9/17). The majority of participants (63%) experienced performing the finger as easy or very easy. Filling the circles correctly was experienced as easy (14/33) or very easy (2/33) by half of the participants and as hard (6/33) or very hard (2/33) by a quarter of the participants.

Comparison of ssDBS and labDBS viral loads

Viral RNA was detected in 33 of 34 ssDBS samples. The negative ssDBS sample corresponded with the negative labDBS sample, with a plasma HCV RNA load of 2.98 log10 IU/mL. In total, 10 HIV-1 positive and 21 HCV RNA positive ssDBS samples were compared to their corresponding labDBS samples. In this analysis, we used the results of ssDBS eluted in L6-buffer, and their corresponding labDBS. The median time between sampling and elution of the ssDBS was 4 days (IQR 2.5–6.5) for HCV RNA positive samples compared to 3 days (IQR 1.5–5) for corresponding labDBS. For HIV-1 RNA positive ssDBS samples the median time between sampling and elution was 5 days (IQR 2–8) compared to 1 day (IQR 1–1.5) for corresponding labDBS. The correlation between ssDBS and labDBS HCV RNA values was high (r = 0.833, Fig 1A). Comparing the HCV viral loads, the Bland-Altman analysis showed that the mean difference of HCV RNA between labDBS and ssDBS was 0.3 log10 IU/mL (95%CI = 0.0, 0.6) and the limit of agreement was between -1.0 to 1.7 log10 IU/mL (Fig 2A).

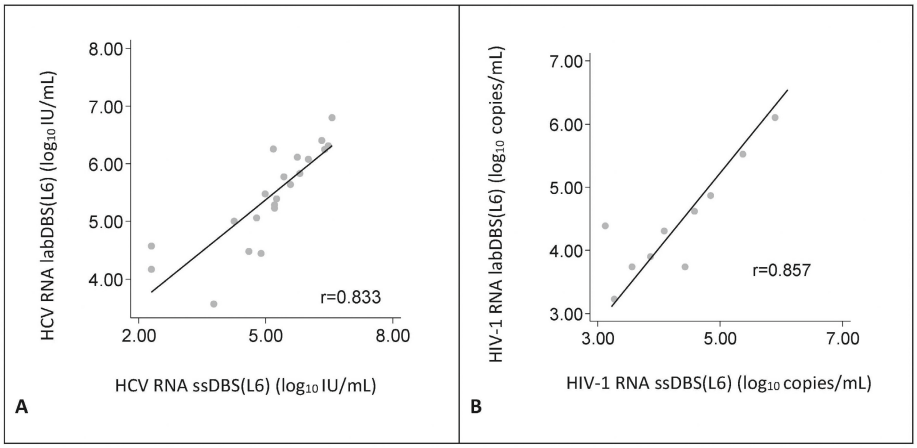

Figure 1. Correlation between self-sampled dried blood spots (ssDBS) and lab-sampled dried blood spots (labDBS) viral load measurements. Individual viral load measurements are plotted (gray dots). The solid line indicates the linear regression line between the two samples. (A) **HCV loads**. The linear relationship between the samples is defined as: log10 IU/mL labDBS HCV = 0.595 x (log10 IU/mL ssDBS) + 2.402. (B) **HIV-1 loads**. The linear relationship between the samples is defined as: log10 copies/mL labDBS HIV-1 = 0.833 x (log10 copies/mL ssDBS) +0.857.

The detection of HCV RNA in ssDBS (eluted in L6-buffer) showed a minimal difference in sensitivity, compared to LabDBS (eluted in L6-buffer). Both methods had high sensitivity: 95.7% (95% CI: 78.1–99.9) for ssDBS and 96.4% (95% CI: 81.7–99.9) for LabDBS.

A strong correlation was also found between ssDBS and labDBS HIV-1 RNA values (r = 0.857, Fig 1B). In the Bland-Altman analysis, the mean difference between labDBS

and ssDBS HIV-1 viral load measurements was 0.1 log10 copies/mL (95% CI = -0.2, 0.5) and the limit of agreement was between -0.8 to 1.1 log10 copies/mL (Fig 2B).

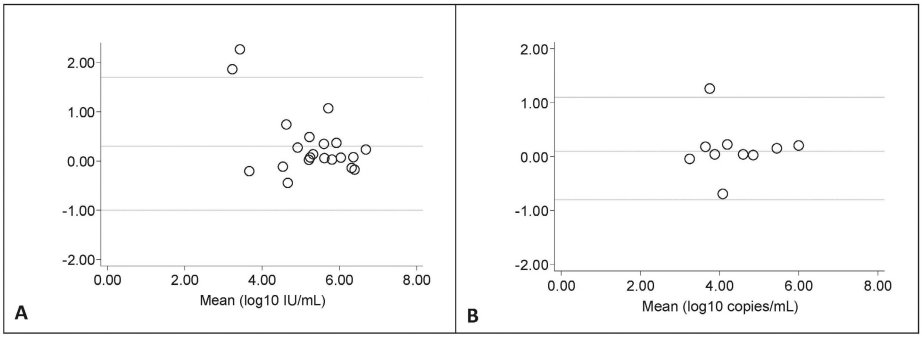

Figure 2. Bland-Altman analysis comparing the labDBS and ssDBS viral load measurements to their mean. The solid line in the middle represents the mean difference between labDBS (laboratory sampled dried blood spots) and ssDBS (self-sampled dried blood spots) viral loads, while the lower and upper lines are for the limits of agreement (± 2 standard deviations). (A)**HCV loads**: 2 values lie outside the limits of agreement; (B) **HIV-1 loads**: one value lies outside the limits of agreement.

The detection of HIV RNA in DBS (eluted in L6-buffer) showed a sensitivity of 100% for both ssDBS (95%: 69.2–100) and LabDBS (95%CI: 73.5–100).

Comparison of plasma and labDBS viral loads

All 39 plasma samples provided quantifiable results, while HCV RNA could not be detected for 1 labDBS sample with a plasma HCV viral load of 2.98 log10 IU/mL. In total 26 HCV positive and 11 HIV-1 positive labDBS samples (eluted in L6) were compared to their corresponding plasma samples. Viral load values measured in labDBS and plasma are shown in Fig 3. A strong correlation was found between labDBS and plasma HCV RNA values (r = 0.958, Fig 3A) with a linear relationship between the samples. The correlation found between labDBS and plasma HIV-1 values was high (r = 0.844, Fig 3B).

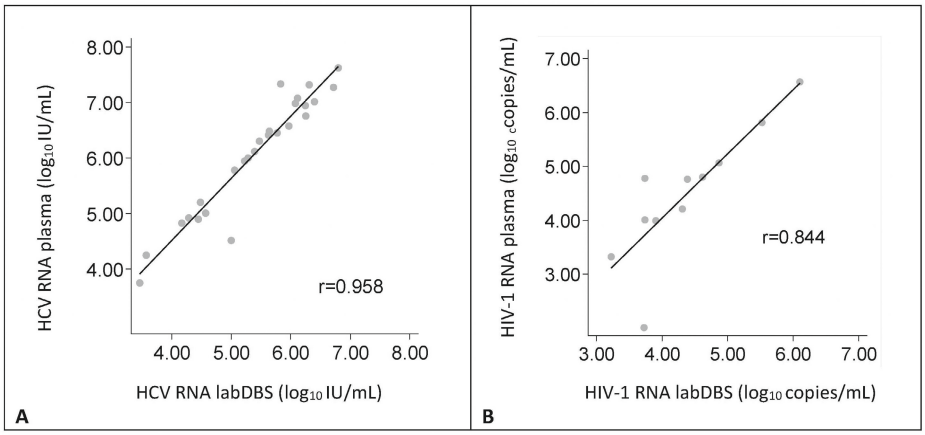

Figure 3. Correlation between laboratory sampled dried blood spots (labDBS) and plasma viral load measurements. Individual viral load measurements are plotted (gray dots). The solid line indicates the linear regression line between the two samples. (A) **HCV loads**. The linear relationship between the samples is defined as: $\log_{10}$ IU/mL plasma HCV = 1.119 x ($\log_{10}$ IU/mL labDBS) + 0.036. (B) **HIV-1 loads.** The linear relationship between the samples is defined as: $\log_{10}$ copies/mL plasma HIV-1 = 1.193 x ($\log_{10}$ copies/mL labDBS) - 0.738.

Evaluation of elution buffers

A total of 36 labDBS samples (25 of HCV RNA positive patients and 11 of HIV-1 RNA positive patients) were eluted in L6-buffer as well as in PBS. In 24 out of 25 labDBS samples, HCV RNA was detected and in 11 labDBS samples, HIV-1 RNA was detected; resulting in 35 quantifiable viral load measurements for comparison.

Viral load measurements in L6-buffer eluted labDBS were compared to their corresponding labDBS samples eluted in PBS (Fig 4). A strong correlation was found between HCV RNA loads measured in L6 and PBS eluates (r = 0.913, Fig 4A). In the Bland-Altman analysis, the mean difference between HCV viral loads measured in PBS and L6 eluates, was -0.7 log10 IU/mL (95% CI = -0.8, -0.5), showing higher viral load results in the L6 eluted samples.

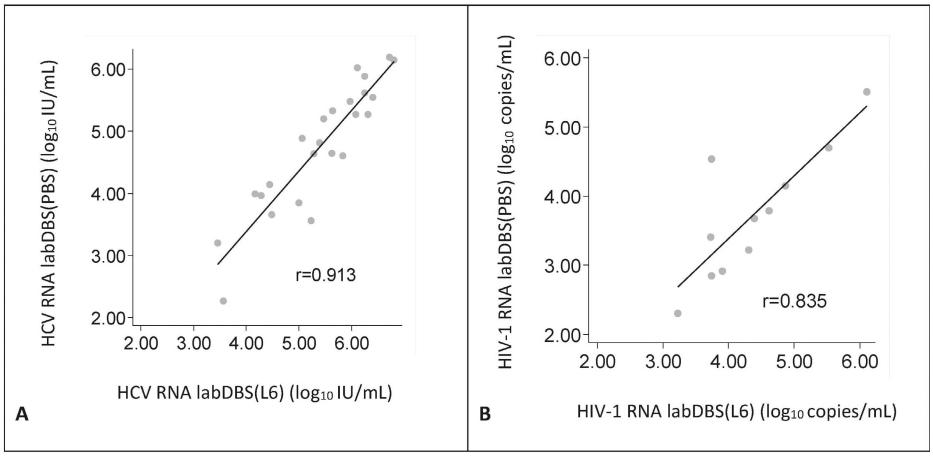

Figure 4. Correlation between viral load measurements of laboratory sampled DBS (labDBS) eluted in L6-buffer and PBS. Individual viral load measurements are plotted (gray dots). The solid line indicates the linear regression line between the two samples. (A) **HCV loads**. The linear relationship between the samples is defined as: log10 IU/mL labDBS (PBS) = 0.978*log10 IU/mL labDBS (L6) –0.534. (B) **HIV-1 loads**. The linear relationship between the samples is defined as: log10 copies/mL labDBS (PBS) = 0.910*log10 copies/mL labDBS (L6)– 0.252. DBS (PBS) = DBS eluted in PBS, DBS (L6) = DBS eluted in L6.

The correlation found between HIV-1 RNA viral loads measured in L6 and PBS-eluates was also high (r = 0.835, Fig 4B). From the Bland-Altman analysis, the mean difference between HIV-1 viral loads in PBS and L6 eluates was -0.6 log10 copies/mL (95% CI = -1.0,-0.3). Again, showing higher viral load results in the samples eluted in the L6 buffer.

RNA stability in DBS

Two to three consecutive viral loads were measured over time of 23 HCV and 8 HIV-1 RNA positive labDBS samples. The samples were stored for a maximum of 21 days at RT after sampling. Samples were measured at t0 (stored <7 days) and/or at t1 (stored 7–13 days) and/or t2 (stored > 14 days). Fig 5 shows the viral load difference between plasma and labDBS plotted over time for each individual sample and the average difference. No statistically significant change in viral load difference was found over the time period up to 21 days, for either HIV-1 or HCV viral loads.

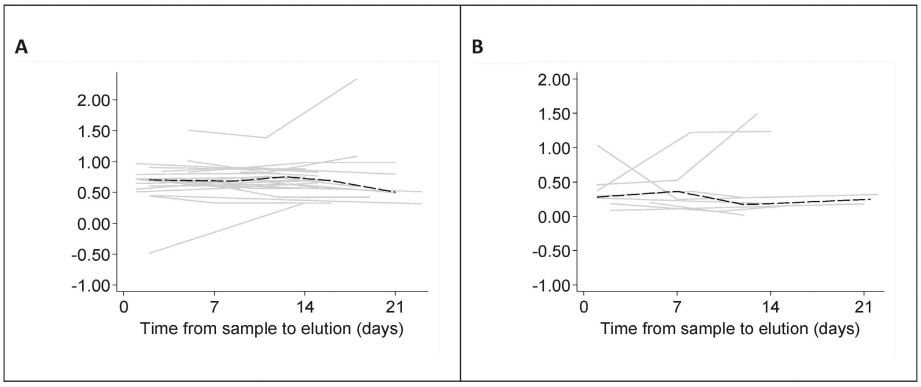

Figure 5. Difference in viral load measurements between plasma and labDBS samples stored for up to 21 days. Viral load measurements of labDBS are plotted over time (gray lines). The black dashed line represents the average difference over time. (A) **HCV loads**: The average change in difference over time is 0.002 log10 IU/mL (95% CI = -0.005, 0.009), p = 0.6. (B) **HIV-1 loads**: The average change in difference over time is 0.004 log10 copies/mL (95% CI = -0.018, 0.026), p = 0.7.

Discussion

We have taken a comprehensive approach to evaluating dried blood spot self-sampling at home for the detection of HCV RNA and HIV-1 RNA. We compared the detection of HCV RNA and HIV-1-RNA in plasma, labDBs, and ssDBS, use of two elution buffers and assessed the quality of ssDBS and user-friendliness of self-sampling. We clearly show that HCV RNA and HIV-1 RNA can reliably be detected in self-sampled DBS. Furthermore, the use of L6 rather than PBS as elution buffer resulted in higher viral load values, in particular for HCV, which suggests that more RNA is eluted, resulting in increased sensitivity compared to PBS as elution buffer. Finally, we showed that HCV and HIV-1 RNA in DBS stored at room temperature are stable for a period of up to 21 days.

Although the majority (97%) of the patients used the paper sampling instructions, and found them helpful, only 36% returned the requested 5 spots with good quality. The main problem we observed with samples of low quality was that the sampled spots were too small. This was unexpected as we had taken the same approach as Hoeller et al., who reported that the large majority (98%) of ssDBS samples were of very good quality [20]. Similar to their approach, we provided patients with both paper and video instructions and assessed samples according to the same quality criteria. The low number of good quality spots in our study may be explained by the fact that about half of the patients did not use the video instructions and some patients experienced performing a finger prick and filling the circles correctly as

(very) hard. Even though a substantial number of the ssDBS samples were not rated as 'good quality', HCV RNA could be detected in all but one ssDBS sample.

To our best knowledge, there are no other reports have been published comparing different buffers to optimise DBS elution protocols for the detection of HCV and/or HIV-1 RNA. Our comparison of two different elution buffers showed that L6 buffer gave more sensitive results with a 0.7 log10 IU/mL mean difference in measured HCV RNA viral loads compared to PBS. This finding has resulted in adjusting the elution protocol of our testing service to the use of L6-buffer and thus lowering the detection limit of the HCV RNA test. The L6-buffer also gave more sensitive results for HIV RNA measurements, but with a 0.6 log10 copies/mL higher level with L6 versus PBS eluted samples.

The utility of DBS as a sampling technique for diagnosis of HCV has been evaluated before and shown to be a good tool for hepatitis C screening and diagnosis [14, 23]. It has been demonstrated that measuring HCV RNA viral loads in DBS is specific and sensitive and strongly correlates with serum HCV RNA measurements [9]. From the data obtained in our study, we also observed that HCV RNA viral loads quantified using labDBS and plasma samples showed strong correlation. Viral loads measured in labDBS were lower than those measured in plasma, while one labDBS sample with a low HCV RNA load gave a negative result. This false negative result can be explained by the reduced sensitivity when using DBS because of the lower input of material. The plasma result of the negative labDBS sample was 3.0 log10 IU/mL, which falls within the range of the previously reported detection limits of 2.2–3.4 log10 IU/mL [8–11]. Similarly, we observed a strong correlation between labDBS and plasma samples for HIV-1 RNA measurements and a decreased sensitivity of DBS compared to plasma, in line with previous studies [12, 24].

As we observed a high agreement between ssDBS and labDBS HCV and HIV-1 RNA viral loads, this study shows, for the first time, that HCV RNA and HIV-1 RNA can adequately be detected in a self-sampled DBS at home. SsDBS can also be used to measure HCV RNA with high sensitivity (96.4%). This result falls in line with a systematic review of the diagnostic accuracy of detecting HCV RNA using DBS, reporting a pooled sensitivity of 98% (95% CI: 95–99) [25].

Furthermore, our data indicate that HCV and HIV-1 RNA stay stable in DBS at RT for up to 3 weeks as RNA levels in DBS measured over time did not change, confirming a previous report [15]. Since time from DBS self-sampling to analysis is approximately 1 to 1.5 weeks, including drying time and transport of the sample by regular mail, this result implies that self-sampled DBS use in a real-life setting is feasible.

Our study had some limitations. As the ssDBS were sampled at home, by patients themselves, we did not know the exact volume of blood applied to the DBS. We did assess this qualitatively, but we did not measure the surface area of the spot. The small difference between labDBS and ssDBS viral load measurements could be caused by a difference in sample volume. Furthermore, we could not assess if patients dried the DBS according to instructions or the time between sampling and posting of the sample. However, by testing the ssDBS and comparing the results to labDBS, we have shown that comparable results can be obtained and thus sampling and posting instructions were assumed to be followed correctly. Although the intended use of the home-based testing service in our project was to diagnose acute infections in MSM at risk of HCV, we did not exclusively include patients with acute HCV infections. One third of the HCV viremic participants had an acute infection. Compared to the chronic phase, where HCV RNA levels tend to remain stable or increase over time [26], early infections are characterised by viral load fluctuations and low HCV RNA levels (<5 log10) [27–29]. Early infections could, therefore, be missed at an early stage of the infection when using the DBS technique. In this study, one patient had a low HCV viral load of 3.0 log10 IU/mL, which could not be detected in DBS. As DBS is less sensitive than a plasma sample test, it is advisable to repeat a DBS test after 2–4 weeks if acute HCV infection is suspected.

The main benefits of ssDBS HCV RNA testing are that DBS sampling is easy and can be performed anonymously at home without the need of any additional resources. This could increase access to testing for the group that does not regularly attend healthcare services, is rarely screened for HCV or does not want to disclose their (sexual) risk behaviour to a health professional. We expect this group to be small in the Netherlands. However, the number of individuals who remain undetected and are unaware of their HCV-status is hard to estimate. We intend to reach these individuals with the NoMoreC project. A home sampling testing service may lower barriers to testing, increase testing frequency and thus limit onward transmission. Takano et al. showed that ssDBS for HIV testing is an acceptable method for MSM in Tokyo and can improve access to testing for MSM who live in rural areas [21]. Coats and Dillon found evidence that DBS may increase the frequency of HCV testing [30]. In addition, the use of DBS-based testing can improve the efficiency of testing in high endemic countries and for hard-to-reach populations. Problems with completing the conventional two-step diagnostic process (serology and HCV RNA confirmatory testing on a follow-up visit) and the asymptomatic nature of early HCV infection are two important factors that hinder early diagnosis [31]. Therefore, simplified diagnostic strategies are urgently needed and should be increased in health facilities and community-based settings. DBS-sampling is well-suited for mobile and outreach testing programmes and has been accepted by WHO as an alternative approach to facilitate access to testing, increase testing rates and reduce loss to follow up [32]. DBS sampling for HCV and HIV diagnosis is also valuable in settings where there is a

lack of access to nearby laboratory facilities for viral load measurements, or where timely delivery of specimens to a laboratory cannot be guaranteed.

In conclusion, self-sampling of DBS at home is a suitable technique for diagnosing HCV and HIV-1 infections. The implementation of this one-step diagnostic approach is feasible and can be used to help scale-up HCV and HIV screening of hard-to-reach, at risk populations as, well as in high endemic countries.

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Chapter 3

Application of the HCV core antigen test to diagnose recently acquired HCV infections among men who have sex with men in the Netherlands

Tamara Prinsenberg, Anders Boyd, Sjoerd Rebers, Maria Prins, Marc van der Valk, Janke Schinkel

Abstract

Introduction
A low cost and sensitive hepatitis C virus (HCV) screening tool is needed to facilitate frequent testing of men who have sex with men (MSM) at risk of HCV infection. It has been previously shown that HCV core antigen (HCVcAg) instead of HCV RNA testing, can be used to diagnose chronic HCV. We studied whether HCVcAg testing could also be used to diagnose recently acquired HCV.

Methods
Medical records from MSM attending the Amsterdam University Medical Centers, with a recently acquired HCV (defined as changing from HCV RNA negative to positive status within a maximum interval of 185 days), between 2003-2018, were reviewed. Stored serum samples from the time at diagnosis of recent infection (primary and reinfections) were tested for HCVcAg. Sensitivity of the HCVcAg assay was calculated, as well as the Pearson's correlation coefficient between HCV RNA and HCVcAg levels. Differences between HCVcAg positive and HCVcAg negative groups were examined.

Results
HCVcAg was detected in 58 out of 64 individuals with detectable HCV RNA levels, resulting in a sensitivity of 90.6% (95%CI = 80.7-96.5%). For recently acquired reinfections, the sensitivity was 80.0% (95%CI = 56.3-94.3%). The correlation between HCVcAg and HCV RNA levels was 0.828 ($p<0.001$). Mean HCV RNA viral load of individuals testing HCVcAg positive was significantly higher than that of HCVcAg negative individuals (5.91 versus 2.90 log10 IU/mL, respectively; $p=0.0016$).

Conclusion
Although the HCVcAg test has a reasonable performance for diagnosing a recently acquired primary HCV infection, sensitivity is not sufficient to allow its use as a screening tool for recent reinfection.

Introduction

In the Netherlands, transmission of hepatitis C virus (HCV) occurs predominantly among men who have sex with men (MSM) [1]. In 2019, 4 years after the introduction of universal access to direct-acting antivirals (DAAs), a 61% decrease in primary HCV incidence and a 79% decrease in HCV reinfection incidence in MSM living with HIV was observed compared to 2015. The incidence of primary infection in this population was 4.1 cases per 1000 person-years, in 2019 [2]. Although the incidence of HCV reinfection in MSM living with HIV showed a slight decrease from 2016 to 2019, it was still high in 2019 at 11 cases per 1000 person-years [2]. Continued efforts are needed to reach HCV micro-elimination in this key population. Early HCV diagnosis, immediate initiation of treatment and behavioral risk reduction strategies may be effective to eliminate HCV in at risk MSM [3]. To facilitate frequent testing of MSM reporting risk behavior associated with HCV infection, an affordable and sensitive HCV screening strategy is needed.

Diagnosis of HCV infection includes the detection of anti-HCV antibodies followed by an HCV RNA test to differentiate between past and ongoing infection. The diagnosis of recently acquired HCV infection is difficult as clinical signs and symptoms are often absent or nonspecific [4]. Furthermore, long periods of time might be required for HCV-specific antibodies to appear, for which a median time of 74 days has been observed from infection to seroconversion in MSM living with HIV [5]. A significant proportion of recently acquired HCV infections may therefore be missed when screening for HCV antibodies alone. In addition, since antibodies remain positive in the majority of individuals following clearance of the primary infection [5], serology is unable to diagnose HCV reinfection. Therefore, an alternative test strategy is needed. An ideal test would be one that directly detects viral RNA or antigen for the early diagnosis of recently acquired infection, both primary and reinfection.

HCV RNA tests are available to diagnose recently acquired HCV, but are costly. Alanine aminotransferase (ALT) levels are often elevated during recently acquired HCV and hence measuring this liver enzyme is often used as an initial screening step. When ALT levels are elevated, HCV RNA is subsequently measured, which greatly reduces costs compared to directly measuring HCV RNA in all individuals at risk. In people living with HIV, 88% experience elevated ALT levels within 3 months of HCV infection [6]. However, ALT levels can remain normal or rapidly normalize after infection, especially during a reinfection and thus this testing strategy will miss some infections [4, 7]. Therefore, further testing for HCV with a more specific marker than ALT is warranted.

A promising alternative to HCV RNA testing for the early diagnosis of recently acquired infection is HCV core antigen (HCVcAg). HCVcAg has been shown to be a

reliable marker and correlates well with HCV RNA levels [8, 9]. A recent meta-analysis showed that HCVcAG testing, instead of HCV RNA testing, can be used for diagnosis of chronic HCV; demonstrating a pooled sensitivity of 98.8% and specificity of 99.0% [10].

The use of HCVcAg to diagnose recently acquired HCV has not been extensively studied. A limited number of studies have measured the performance of HCVcAg testing for recently acquired infection, showing an 87-89% sensitivity [11, 12]. The objective of this study was then to determine if HCVcAg can be used for the diagnosis of recently acquired HCV infection during routine care of MSM at risk of HCV infection in Amsterdam.

Materials and Methods

Study design and population

We conducted a retrospective chart review of electronic medical records (EMRs) from MSM who had received medical care for recently acquired HCV infection at the Amsterdam University Medical Center (AUMC), location University of Amsterdam, from January 2003 to December 2018.

For the present study, we included individuals if they were MSM, aged 18 years or older, had a recently acquired HCV (defined as changing from HCV RNA negative to positive status within a maximum interval of 185 days), and had a stored serum sample available from the time of HCV diagnosis.

Study procedures

We collected the following demographic and clinical data from patient EMRs, which were obtained closest to the date of first positive HCV RNA result: age, HCV RNA viral load, type of infection (primary or reinfection), HCV genotype, HIV status (anti-HIV antibody positive or negative) and ALT levels. We defined ALT as elevated when its value was >55 U/L. We also obtained data on HIV viral load and CD4+ cell count for individuals with HIV and on current PrEP use for individuals without HIV. HIV viral loads were defined as undetectable when HIV RNA level was <200 copies/mL. We distinguished between primary HCV infection and reinfection by a negative and positive anti-HCV antibody test, respectively, prior to diagnosis of recently acquired HCV infection.

Blood serum samples were tested for HCVcAg using the Architect HCV core antigen assay (Abbott Laboratories, Abbott Park, IL, USA) with a lower limit of detection of 3 fmol/L. Quantitative HCV RNA testing was performed using the CAP/CTM assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Almere, the Netherlands)

or the branched DNA assay (bDNA, Versant, Siemens Healthcare Diagnostics, the Hague, the Netherlands). Qualitative HCV RNA measurements were performed using the Siemens Versant HCV transcription-mediated amplification test (TMA assay). All assays were performed according to the manufacturer's instructions. HCV genotype was determined by sequencing and phylogenetic analysis of the NS5B region. For individuals with missing data on ALT levels, ALT was measured using the stored blood samples at the time of HCV diagnosis.

Statistical analyses

Demographic and clinical data were presented as median (IQR) and proportions. Differences between HCVcAg positive and HCVcAg negative groups were statistically compared using Fisher's Exact test for categorical variables and Kruskal-Wallis test for continuous variables.

Data of individuals with quantitative HCV RNA results were used to measure linear correlation between HCV RNA and HCVcAg values. HCV RNA and HCVcAg levels were log10-transformed, plotted and compared using Pearson's correlation coefficient. A linear regression model was then fit using log10-transformed levels of HCVcAg as the dependent variable and log10-transformed levels of HCV RNA as the independent variable.

To determine whether there were any substantial departures in measurements from HCV RNA and HCVcAg, we conducted a Bland-Altman analysis. Only data from individuals with quantitative HCV RNA results were used. This analysis involves plotting the difference between HCV RNA and HCVcAg levels against their means. Limits of agreement (LOA) are defined as ±2 standard deviations (SD) of the mean difference, while any value below the lower limit or above the upper limit defines an outlier. This analysis does however require that the assay measure the same quantity. To resolve this issue, HCV RNA and HCVcAg levels were standardized so that both their means and SD were equal to 0 and 1, respectively.

To evaluate the predictive capacity of HCVcAg against another marker of HCV activity, we calculated the sensitivity of a positive HCVcAg result and elevated ALT levels (i.e., >55 U/L) as predictors of recently acquired HCV infection, recently acquired primary HCV infection and recently acquired HCV reinfection. Since we only had positive HCV RNA individuals, we were unable to calculate specificity and positive and negative predictive values for this study. We compared sensitivities of predictors using McNemar's test: a paired relative test of sensitivity [13].

Statistical analysis was conducted using STATA 15.1 (STATA Corporation, College Station, Texas, USA) and a p-value <0.05 was considered statistically significant.

Results

Description of sample selection

From January 2003 to December 2018, we identified 169 new HCV infections among MSM. The flow of sample selection is described in Figure 1. Eighty-nine infections were a chronic HCV infection at the time of diagnosis. In 13 of the 80 remaining recently acquired HCV infections, no stored blood serum sample was available of the time of diagnosis and 3 gave an invalid HCVcAg result. A total of 64 HCV infections were then included in the study, of which 44 were a primary infection and 20 a reinfection.

Characteristics of the study population

The study population contained 52 individuals, having a total of 44 primary infections and 20 reinfections among them. Table 1 shows the number of infections per individual. If data of more than one infection of the same individual were used, they were analysed as separate data files; resulting in a total population of 64.

Table 1. Distribution of the type of infections among the study population

	Number of individuals	Type of infection(s)/ individual		Primary infections included	Reinfections included
	37	Primary	-	37	-
	6	Primary and	1 reinfection	6	6
	1	Primary and	2 reinfections	1	2
	6	-	1 reinfection	-	6
	1	-	2 reinfections	-	2
	1	-	4 reinfections	-	1
Total	**52**			**44**	**20**

Data of 52 individuals having a total of 64 recently acquired HCV infections were included in the study: 44 primary infections and 20 reinfections.

Of included individuals, median age was 44 years (interquartile range [IQR] 40-49.5) and all but one individual were living with HIV (Table 2). HIV viral load was detectable (i.e., HIV RNA ≥200 copies/mL) in 13 of 63 individuals with HIV (20.6%). The only individual without HIV was using PrEP. Median CD4 count was 475 cells/mm^3 (IQR 380-665). The HCV genotype distribution was as follows: genotype 1a, 68.8%; genotype 1b, 4.7%; genotype 2b, 3.1%; genotype 3a, 1.6%; and genotype 4d, 21.9%.

Ninety-one percent (*n*=58/64) of the samples diagnosed with recently acquired HCV tested positive for HCVcAg. Individuals who tested positive for HCVcAg had a significantly higher HCV RNA viral load (Table 2). There was no other difference found in any of the measured characteristics between individuals testing positive

versus negative for HCVcAg (Table 2). Multivariable analysis was precluded by the few characteristics demonstrating differences between HCVcAg positive and negative individuals.

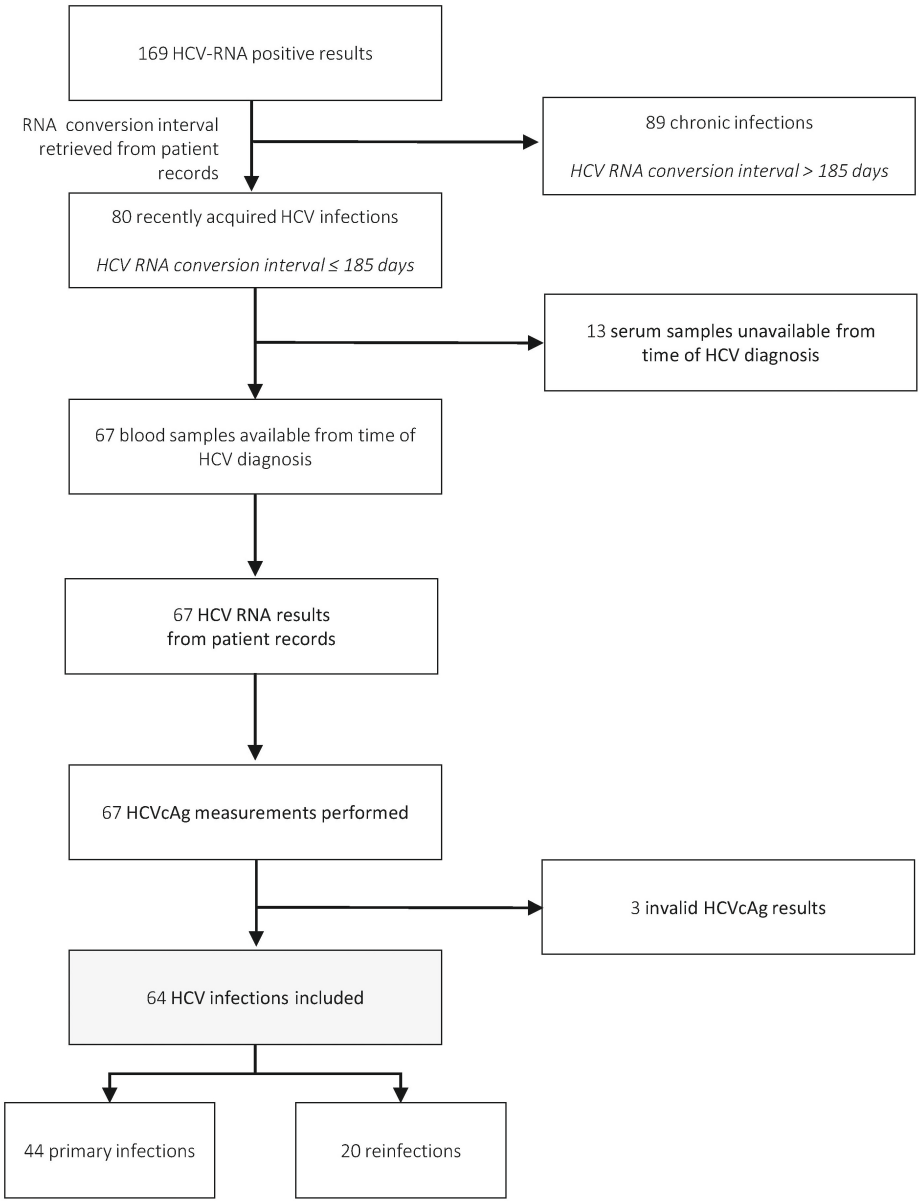

Figure 1. Flowchart of data gathered for the study from individuals attending care at the AUMC from 2003-2018.

Table 2. Characteristics of the study population according to hepatitis C virus core antigen (HCVcAg) status

Characteristic (*n*=64)	Total	Negative (≤3 fmol/mL)	HCVcAg status		*p**
			Positive (>3 fmol/mL)		
		(*n*=6)	(*n*=58)		
Median (IQR) HCV RNA, log_{10} IU/mL[§]	5.68 (3.96-6.46)		2.90 (2.63-3.00)	5.91 (4.95-6.55)	0.0016
Type of infection					0.071
Primary	44 (68.8)		2 (33.3)	42 (72.4)	
Re-infection	20 (31.3)		4 (66.7)	16 (27.6)	
HCV genotype					0.632
1a	44 (68.8)		6 (100)	38 (65.5)	
1b	3 (4.7)		0	3 (5.2)	
2b	2 (3.1)		0	2 (3.5)	
3a	1 (1.6)		0	1 (1.7)	
4d	14 (21.9)		0	14 (24.1)	
Median (IQR) ALT, U/L	178 (56-403)		176 (22-322)	186 (62-420)	0.434
Elevated ALT levels (>55 U/L)	48 (75.0)		4 (66.7)	44 (75.9)	0.635
Median (IQR) age, years	44 (40-49.5)		44.5 (39-61)	44 (41-49)	0.661
HIV-diagnosis	63 (98.4)		6 (100)	57 (98.3)	1.000
HIV viral load[‡]					0.330
Detectable[‡] (≥200 copies/mL)	13 (20.6)		0	13 (22.8)	
Undetectable [‡] (<200 copies/mL)	50 (79.4)		6 (100)	44 (77.2)	
Median (IQR) CD4+ cell count, /mm^3[‡]	475 (380-665)		580 (440-640)	470 (350-670)	0.252
CD4+ cell count[‡]					0.468
<200/mm^3	1 (1.7)		0	1 (1.9)	
200-500/mm^3	31 (51.7)		2 (33.3)	29 (53.7)	
>500/mm^3	28(46.7)		4 (66.7)	24 (44.4)	
PrEP use[¶]	1 (100)		N/A	1 (100)	*ntp*

Data are from individuals with recently acquired HCV infection seeking care at the Amsterdam University Medical Centers, location University of Amsterdam, from 2003-2018. All statistics given are n (%) unless otherwise stated.

*Differences between HCVcAg negative and positive samples were statistically tested using Fisher's Exact test for categorical variables and Kruskal-Wallis test for continuous variables.

[§]Only for samples with quantitative HCV RNA result (n=45)
[‡]Only for samples of individuals with HIV (n=63).
[¶]Only for sample of individual without HIV (n=1).
Abbreviations: ALT, alanine transaminase; HCV, hepatitis C virus; HCVcAg, hepatitis C virus core antigen; HIV, human immunodeficiency virus; IQR, interquartile range; IU, international units; N/A, not applicable; ntp, no test performed; PrEP, pre-exposure prophylaxis for HIV infection.

Comparison of HCV RNA and HCVcAg levels

The Pearson's correlation coefficient for $\log_{10}$ transformed values of HCV RNA and HCVcAg levels of 45 individuals was 0.828 (p<0.0001) (Figure 2).

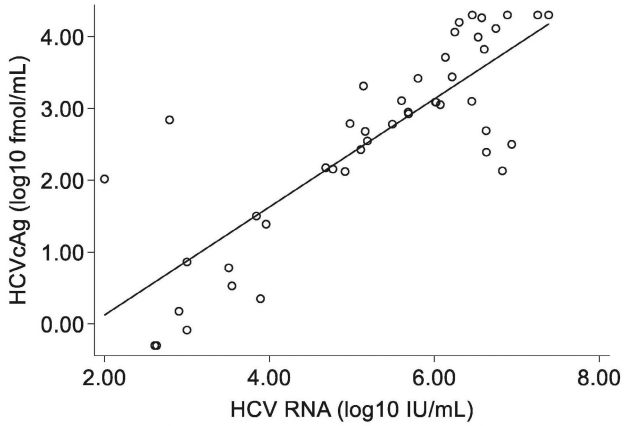

Figure 2. Relationship between hepatitis C virus RNA and core antigen levels
Data are from individuals (n=45) with recently acquired HCV infection seeking care at the Amsterdam University Medical Centers, location University of Amsterdam, from 2003-2018. The linear relationship between $\log_{10}$-transformed levels of HCV RNA and HCV core antigen (HCVcAg) is depicted in by a solid line and is defined as: $\log_{10}(\text{HCVcAg}) = 0.911*\log_{10}(\text{HCV RNA}) + 2.914$.

The Bland-Altman analysis showed that the mean difference between standardized levels was 0 (95%CI = -0.176 – +0.176), which was expected given that the measures were standardized to have a mean of 1. The limit of agreement was between -1.173 and 1.173 (Figure 3). Four values were outside the limits of agreement and were thus identified as outliers. Characteristics of the individuals representing the four outliers are given in Table 3. Three out of four outliers were individuals with a reinfection. There were no other noteworthy characteristics of these individuals compared to the overall study population.

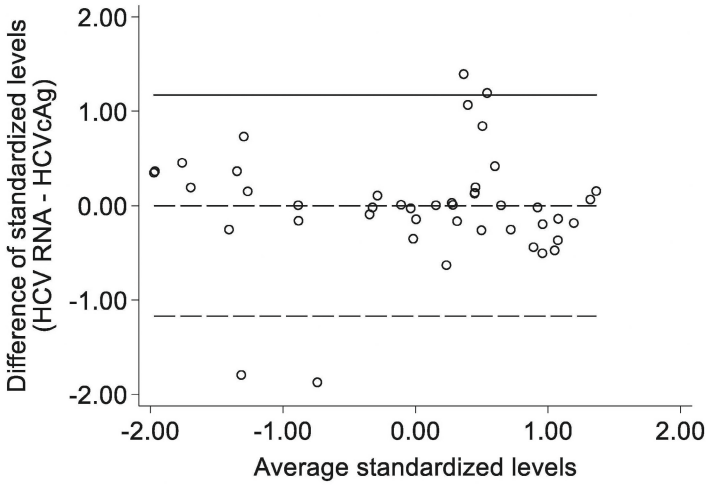

Figure 3. Bland-Altman analysis comparing standardized hepatitis C virus RNA and core antigen levels to their mean. Data are from individuals (n=45) with recently-acquired HCV infection seeking care at the Amsterdam University Medical Centers, location University of Amsterdam, from 2003-2018. The solid line in the middle represents the mean difference between standardized levels of HCV RNA and HCV core antigen (HCVcAg), while the lower and upper lines are for the limits of agreement (±2 standard deviations of the mean difference).

Table 3. Description of individuals with values outside the limits of agreement of the Bland-Altman analysis

Characteristic	Patient 10	Patient 48	Patient 49	Patient 60
HCV RNA (log_{10} IU/mL)	6.94	6.82	2.79	2.00
HCVcAg (fmol/mL)	316.5	134.92	691.18	104.27
Primary or re-infection	Re-infection	Re-infection	Primary	Re-infection
HCV genotype	1a	1a	1a	3a
ALT (U/L)	247	62	81	385
Age (years)	44	50	33	31
HIV status	Positive	Positive	Positive	Positive
HIV RNA (copies/mL)	Undetectable	Undetectable	Undetectable	Undetectable
CD4+ cells/mm^3	480	390	350	960
PrEP use	N/A	N/A	N/A	N/A

Sensitivity of positive HCVcAg and elevated ALT as predictors of recently acquired HCV

The sensitivity to predict recently acquired HCV was 90.6 % (95%CI = 80.7-96.5%) for a positive HCVcAg result and 75.0% (95%CI = 62.6-85.0%) for elevated ALT. The relative ratio comparing the sensitivities between HCVcAg and elevated ALT as

predictors of recently acquired HCV was 1.21 (95%CI = 1.03-1.42), demonstrating HCVcAg to be a slightly more sensitive predictor (p=0.03).

For recently acquired primary infections, the sensitivity was 95.5% (95%CI = 84.5-99.4%) for a positive HCVcAg result and 79.5% (95%CI = 64.7-90.2%) for elevated ALT. The relative ratio comparing the sensitivities between HCVcAg and elevated ALT as predictors of a primary HCV was 1.20 (95%CI = 1.06-1.36), demonstrating HCVcAg to be a slightly more sensitive predictor (p=0.03).

For recently acquired reinfections, the sensitivity was 80.0% (95%CI = 56.3-94.3%) for a positive HCVcAg result and 65.0% (95%CI = 40.7-84.6%) for elevated infections. The relative ratio comparing the sensitivities between HCVcAg and elevated ALT as predictors of a HCV reinfection was 1.23 (95%CI = 0.97-1.55), while there was no significant difference in sensitivities between predictors (p=0.26).

Discussion

The current study demonstrates that HCVcAg has good diagnostic performance for the diagnosis of recently acquired HCV among MSM in the Netherlands, with a sensitivity of 90.6% (95%CI = 80.7-96.5%). In line with previous studies, we found that HCVcAg levels correlate well with HCV RNA levels [8, 9] and the mean HCV RNA viral load of individuals who tested positive for HCVcAg was significantly higher than that of those who tested HCVcAg negative [11, 12].

The use of HCVcAg to diagnose chronic HCV among people living with and without HIV has been previously assessed, including during DAA treatment response monitoring and its cost-effectiveness [14, 15]. In our study, we build on these previous findings by assessing the application of the HCVcAg assay for the diagnosis of recently acquired HCV among MSM. The studies in individuals with chronic HCV infection have demonstrated that the use of HCVcAg testing was a cost-saving strategy for the diagnosis of chronic HCV infections, on-treatment monitoring, but was inadequate to establish SVR. A systematic review concluded that the HCVcAg test can achieve similar diagnostic accuracy to HCV RNA for the identification of active HCV when the viral load exceeds 3000 IU/mL (3.48 log10 IU/mL) [16]. Our results corroborate this evidence, as the mean viral load of individuals with a negative HCVcAg test result was 2.90 (IQR: 2.63-3.00) log10 IU/mL. Even though the diagnostic performance of the HCVcAg test is insufficient in individuals with a low HCV viral load, it could be used as a screening tool to monitor MSM at risk of HCV if there are limited financial resources, as the price of the HCVcAg test is more affordable (€32) than HCV RNA (€105-€225). The HCVcAg test could be applied to screen every three months at the same cost as using HCV RNA once per year. More

frequent testing will most likely diagnose recently acquired infection at an earlier stage than with annual testing. However, we do need to emphasize that a proportion of MSM with a recently acquired HCV infection will be missed (9.4%) when using the HCVcAg test. Therefore, if HCV screening is not subject to budgetary constraint it is preferred to use an HCV RNA assay, which is more sensitive and can detect viral loads as low as 1.18 Log10 IU/mL. Also, it has to be kept in mind that the HCVcAg test requires a lot of a laboratory technician's time, which in turn results in higher overall costs.

In the Netherlands, MSM living with HIV are screened for recently acquired HCV by means of testing for ALT twice a year [17]. MSM living with HIV who engage in behaviors associated with a high risk of HCV infection are additionally tested yearly using HCV serology. If ALT levels are elevated or HCV antibodies are detected, HCV RNA testing is performed. In our study population, by using ALT alone, 23.8% (15/63) of recently acquired HCV infections would have been missed. By screening with HCVcAg instead of ALT, the number of missed infections would decrease to 9.3% (6/64). A study from Vanhommerig et al., evaluating HCVcAg for HCV screening among MSM living with HIV, detected HCVcAg in 100% of MSM with a recent infection, and elevated ALT in only 20% [18]. Although the number of MSM with a recent infection was small (n=5), their results also indicated HCVcAg to be a better screening tool than elevated ALT. Our comparison of sensitivities showed that HCVcAg is a slightly more sensitive predictor of HCV infection than elevated ALT. A Dutch modelling study by Popping et al. demonstrated that a HCVcAg monitoring strategy targeted only at a group of previously HCV-infected MSM living with HIV (high-risk group), while the rest of MSM living with HIV is monitored with ALT twice a year, is less costly compared with the current monitoring approach and will result in a reduction of HCV incidence and prevalence [19]. The model was based on a sensitivity of elevated ALT of 70-100% and HCVcAg of 90-100% [19]. Yet, we found no significant difference in sensitivity between HCVcAg and elevated ALT in the high-risk group of individuals with a reinfection and we would therefore not expect an impact on HCV incidence by replacing ALT by HCVcAg in this group. Replacing ALT by HCVcAg in the group of MSM who have not been previously infected can be a good strategy that may impact the HCV incidence and prevalence as we found HCVcAg to be a significantly more sensitive predictor of a recently acquired primary infection than elevated ALT, with sensitivities of 95.4% and 79.5% respectively.

For MSM without HIV who use PrEP, the current Dutch PrEP guidelines recommend screening for anti-HCV antibodies once a year [20]. If antibodies are detected, the infection is then confirmed using an HCV RNA test. Because of the delay in HCV antibody seroconversion, antibody screening alone has the potential to miss early HCV infections. For individuals who have previously acquired HCV, PrEP guidelines recommend yearly HCV RNA screening [20]. Unfortunately, our study included only

one individual on PrEP and thus our inference on the use of HCVcAg testing for this group is limited. Nonetheless, it is likely that the use of HCVcAg instead of antibodies would improve HCV screening among MSM on PrEP for primary infections. This idea is supported by a recent study from Gras et al. in which the majority (89%) of MSM on PrEP with a recently acquired HCV infection tested HCVcAg positive, even before the detection of HCV antibodies and despite the fact that individuals were asymptomatic and only 25% had elevated ALT [21].

In addition to a sensitive and cost-effective test, regular assessment of HCV risk and innovative testing approaches can improve the earlier detection of recently acquired HCV infection. The use of the MOSAIC questionnaire, a validated risk-score, based on self-reported known risk factors can easily identify the most at-risk individuals [22]. For individuals who reported recent high-risk sexual practices or other risk factors associated with HCV infection, more frequent testing should be considered by the treating physician. Offering home-based testing services gives more accessible testing, particularly to MSM who are then able to control their own health. We previously showed that an online home-based testing service, using self-sampled dried blood spots (DBS) sent to a central laboratory for HCV RNA testing, was considered acceptable and easy to use by most MSM [23]. The self-collection of capillary blood for a testing approach where the sample is first tested for ALT and HCVcAg and only tested for HCV RNA if ALT is elevated, can be a good alternative. HCVcAg can be measured in DBS to detect active HCV with a sensitivity of 90.7% [24], but to the best of our knowledge there are no reports of ALT measurements in DBS. Strategies to measure ALT in DBS would have to be investigated or the use of an alternative sampling method, such as the self-collection of capillary blood into Microtainer Microtubes. Ansari et al. found that liver function tests, including ALT, can be measured from capillary blood collected in Microtainer Microtube, and were comparable to plasma venous samples [25]. The home collection of the blood sample demonstrated good patient usability.

One limitation of this study is the absence of quantitative HCV RNA results for 19 individuals in our study population, which could have increased type II error for many of the analyses. Nevertheless, we were still able to establish a significant correlation between HCVcAg and HCV RNA levels and to observe a significant difference between HCV RNA viral loads for individuals who tested HCVcAg positive versus HCVcAg negative. Another limitation is that no cost-benefit analysis on implementing the HCVcAg assay to identify recently acquired HCV infection was performed. The costs and benefits of replacing HCV RNA with HCVcAg tests, using HCVcAg as a screening tool based on self-reported risk and the use of HCVcAg DBS home-based self-sampling test will certainly be useful to inform HCV testing strategies for different groups (e.g., MSM at high risk of HCV infection, MSM with a previous HCV infection, MSM on PrEP, MSM living with HIV). However, given the

lower costs of HCVcAg compared to HCV RNA, the use of HCVcAg would probably be more cost-effective for the diagnosis or exclusion of recently acquired HCV infection in MSM engaging in behaviors associated with HCV. More studies are recommended to better inform the use of HCVcAg in the group of MSM with a previous HCV infection. Increasing the test frequency (e.g. every 3 months) in this group may be needed.

In conclusion, the HCVcAg assay has reasonable diagnostic performance and its use has the potential to lead to some cost savings in diagnosing recently acquired primary HCV infection. More frequent testing of MSM at high-risk for HCV infection is recommended to diagnose HCV infection at an earlier stage and subsequently initiate treatment, which will help prevent HCV transmission and reduce HCV incidence. The HCVcAg test could be a good addition to the current monitoring approach to increase testing frequency for MSM who have not previously had an HCV infection. The sensitivity of the HCVcAg test is not sufficient to allow its use as a screening tool for recent reinfection.

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Chapter 4

Design and implementation of a multilevel intervention to reduce hepatitis C transmission among men who have sex with men in Amsterdam: co-creation and usability study

Tamara Prinsenberg, Paul Zantkuijl, Wim Zuilhof, Udi Davidovich, Janke Schinkel, Maria Prins, Marc van der Valk

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Abstract

Background
In the Netherlands, transmission of hepatitis C virus (HCV) occurs primarily among men who have sex with men (MSM). Early HCV testing of at-risk MSM and immediate initiation of treatment will prevent onward transmission, but this may not be sufficient to eliminate HCV in a population with ongoing risk behaviors. Therefore, targeted socio-culturally acceptable preventive measures, including behavioral interventions, are urgently needed. Currently, little contextually appropriate information about HCV or risk reduction interventions is available.

Objective
The objective of this project was to develop an intervention to reduce HCV transmission among MSM in Amsterdam through a co-creation process, with the input of men from the targeted community directly impacting intervention content, design, and implementation.

Methods
We developed a multilevel intervention targeting 6 levels: individual, community, professional, context, patient, and network. The intervention was developed in close cooperation between health professionals, gay community members, commercial stakeholders, and stakeholders from within the gay community. The co-creation process had 4 phases: a needs assessment, stakeholder engagement, co-creation, and implementation. The co-creation phase continued until consensus was reached between the researchers and community members on the intervention content and design. The final intervention, NoMoreC, was completed within 2 years, and implementation started in February 2018.

Results
NoMoreC includes web-based and face-to-face components as well as an anonymous HCV testing service. The NoMoreC website provides information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options, and partner notification. The face-to-face component comprises a risk reduction toolbox, training for health professionals, and providing tailored advice to sex on premises venues. NoMoreC is promoted by an active voluntary campaign team.

Conclusions
Involving the community and stakeholders in the creation of NoMoreC has been the main strength of this project. It has resulted in an intervention with various components that resonates with the gay community at risk of HCV infection. The uptake and acceptability of the described intervention will be evaluated in the future. The description of the co-creation process and implementation of the project

may serve as a rich and useful source for others who want to develop culturally and context appropriate HCV interventions.

Introduction

Hepatitis C virus (HCV) infection is a major public health problem: An estimated 71 million people worldwide are living with chronic HCV infection, which, if left untreated, may progress to serious liver disease [1]. In 2016, approximately 399,000 people died from HCV-related cirrhosis and liver cancer, and the number of deaths increases each year [2]. In the Netherlands, HCV transmission occurs primarily among HIV-positive men who have sex with men (MSM), as HCV incidence dropped to nearly zero among people who inject drugs [3-5]. Since 2000, there has been an unexpected and substantial increase in HCV incidence among HIV- positive MSM globally [6,7]. Data from the international CASCADE collaboration demonstrated a significant overall increase in HCV incidence among HIV-positive MSM — from 0.07/100 person-years in 1990 to 1.8/100 person-years in 2017 [8]. The incidence of HCV reinfection among HIV-positive MSM is 3-10 times higher than the primary infection incidence, and more recent data show ongoing HCV transmission among HIV-negative MSM using pre-exposure prophylaxis [9-14].

Recent improvements in HCV therapy have resulted in multiple highly tolerable direct-acting antiviral (DAA) regimens with cure rates of over 95% [15]. In many countries, DAA therapy is available for patients with chronic HCV [16]. In the Netherlands, unrestricted access to DAA for all chronically HCV-infected individuals has been available since 2015. The uptake of HCV treatment in HIV/HCV co-infected individuals in the Netherlands has been very rapid: 15 months after the introduction of DAA, 83% of HIV/HCV co-infected MSM were cured [17].

Early testing of at-risk MSM and immediate initiation of treatment prevent onward transmission, but this may not be sufficient to eliminate HCV in a population with ongoing risk behavior [18-20]. Uptake of DAAs differs greatly between countries [21], and international HCV transmission in HIV-positive MSM persists [22,23]. Salazar-Vizcaya et al [18] showed that prevention of high-risk behavior alone could result in a considerable reduction of HCV transmission among HIV-infected MSM based on different modeled scenarios on hypothetical behavioral and treatment interventions [18]. In another modeling study, the potential impact of the Swiss HCVree Trial was assessed [24]. In this trial, a behavioral intervention that prevents HCV transmission through individual risk counseling was combined with early DAA treatment. The model suggested that treatment plus counseling can reduce HCV prevalence among HIV-positive MSM. These modeling studies indicate that early treatment in combination with the implementation of health promotion interventions that reduce

high-risk behavior is an effective strategy to control the HCV epidemic in MSM. Targeted socio-culturally acceptable preventive measures, including behavioral interventions for MSM at risk of HCV infection, are scarce and urgently needed.

In 2016, we established the Amsterdam MSM Hepatitis C Free (MC Free) consortium with the goal of stopping HCV transmission among MSM in Amsterdam. MC Free is the driving force behind the NoMoreC project, an innovative project co-created with the Amsterdam gay community. Actively involving the target group in the development and implementation of an intervention is a promising approach to increase engagement of the target population [25]. This asks for strong collaboration between healthcare professionals, researchers, and the target group. Using a co-creational approach ensures that the design is pragmatic, local, and tailored to the target group and the specific settings for which it is created. Consequently, co-creation results in contextually appropriate intervention strategies as well as creation of a platform for co-learning and enhancing ownership and empowerment among the target group [26,27].

For the NoMoreC project, a multilevel intervention was developed and implemented at the individual, community, healthcare professional, context, patient, and network levels. We believe that targeting these levels simultaneously will lead to a more substantial impact on risk reduction behavior, testing frequency, early diagnosis, early treatment, and partner notification than a single-level intervention would. In this paper, we describe the co-creation process including the development and implementation of the various components of the multilevel intervention.

Methods

MC Free Consortium

In January 2016, the Public Health Service of Amsterdam (PHSA), Amsterdam University Medical Centers, location Academic Medical Center, Soa Aids Nederland, and Amsterdam Institute of Global Health partnered to establish the Amsterdam MSM Hepatitis C (MC Free) consortium. Soa Aids Nederland is a nongovernmental organization specializing in sexual health and sexually transmitted infections (STIs) with strong links with the gay community. MC Free is advised by an international scientific advisory board and a local community advisory board. The consortium combines the knowledge and expertise of professionals from different backgrounds and members of the gay community to work towards a common goal: the elimination of HCV among MSM in Amsterdam.

Multilevel Intervention

MC Free developed the NoMoreC project, a multilevel intervention strategy targeting 6 levels: individual, community, professional, context, patient, and network. For each level, we formulated specific aims (Table 1).

Co-Creation Process

The NoMoreC project was developed in close cooperation with health professionals and the gay community in Amsterdam. Commercial stakeholders and stakeholders from within the gay community were involved at an early stage. The process had 4 distinct phases: a needs assessment, stakeholder engagement, co-creation, and implementation phase (Figure 1).

Needs Assessment

In January 2016, prior to development of the intervention, we conducted 2 focus group discussions to identify and discuss the needs regarding hepatitis C information and testing options. MSM at risk of HCV infection were recruited through MSM cohorts of the PHSA, 4 HIV treatment centers in Amsterdam, the Dutch HIV patient association, a magazine for people living with HIV, and a gay dating site. One focus group was held with 7 HIV-positive MSM who had been HCV-infected in the past and 1 HIV-positive MSM who was under HCV treatment at the time of the focus group discussion. The participants of the second focus group were HIV-positive MSM who had never been HCV-infected but had concerns about becoming infected. The following themes were discussed in both focus groups: hepatitis C information (need and current availability), hepatitis C risk, concerns of getting (re)infected, desired hepatitis C prevention tools, motivators and barriers for risk reduction strategies during sex, and different hepatitis C testing options. Structured questions were formulated and posed to the groups, touching upon all themes. The themes were expanded upon depending on the group dynamics. Responses were categorized and clustered per theme.

In addition, 3 intervention ideas were suggested by the researchers: (1) home-based HCV testing service, (2) checklist to estimate personal risk of contracting hepatitis C, and (3) toolbox containing items to assist in HCV risk reduction. The feedback and opinions of the participants regarding these suggestions were collected and processed later in the further development of these interventional aspects.

Table 1. Levels and corresponding project aims and target groups of the NoMoreC project.

Level	Aims	Target group or context
Individual level	Increase HCV[a] knowledge and awareness, promote regular testing, and promote risk reduction behavior	MSM[b] at risk of contracting hepatitis C, including MSM with high-risk sexual behavior and MSM who have sex in networks where hepatitis C infections occur
Community level	Increase HCV knowledge and awareness, promote regular testing, promote risk reduction behavior, and create an atmosphere where men feel responsible for keeping their community HCV-free	Gay community
Professional level	Increase HCV knowledge and awareness, increase knowledge about the available HCV testing options, increase knowledge about risk reduction strategies, and improve partner notification	Healthcare professionals who give care to MSM at risk of hepatitis C, including professionals at the PHSA[c] STI[d] clinic, professionals at HIV treatment centers, and GPs[e] with a substantial number of MSM attending their practices
Context level	Create an enabling environment for risk reduction[f]	Sex on premises venues and organizers of sex parties
Patient level	Provide fast linkage to care and prevent reinfection	MSM who acquired HCV
Network level	Penetrate social and sexual networks where hepatitis C infections occur and enhance partner notification	Social and sexual networks where hepatitis C infections occur

[a]HCV: hepatitic C virus; [b]MSM: men who have sex with men; [c]PHSA: Public Health Service of Amsterdam; [d]STI: sexually transmitted infection; [e]GPs: general practitioners; [f]An enabling environment is an environment where risk reduction is facilitated and products for risk reduction are available.

Stakeholder Engagement

Based on the needs assessment, a first concept of the NoMoreC multilevel intervention was designed and presented to a group of commercial and gay community stakeholders in September 2017. This group consisted of owners or managers of sex on premises venues (SOPV), gay chat and dating sites, fetish shops, (sex) party organizers, and representatives of HIV interest groups and organizations active in the gay or HIV community. In October 2017, we organized a second stakeholder meeting for professionals who either treat HCV-infected individuals or are involved in the prevention and control of HCV infections. The meeting was attended by nurses, nurse practitioners, and physicians from HIV treatment centers and STI clinics in Amsterdam; policy advisors and program managers from the

National Institute for Public Health and Environment; and Soa Aids Nederland. A total of 9 commercial stakeholders, 4 gay community stakeholders, and 31 professionals attended the meetings. Furthermore, presentations were organized for the clinical staff of HIV treatment centers in Amsterdam. The goal of the stakeholder meetings and presentations was to introduce the NoMoreC intervention and ask for input on the development and implementation of the project as well as to strengthen relationships with stakeholders. Input given during the meetings was used to adjust the concept NoMoreC intervention.

Co-Creation Phase

The first co-creation meeting with members of the gay community (n=11) to shape the NoMoreC project was organized in July 2017 prior to the stakeholder meetings, where the first concept intervention was presented. An intensive co-creation phase followed from November 2017 until February 2018 with a group of men from the Amsterdam gay community at risk of HCV or men who had been HCV-infected. The co-creation phase was an iterative process with co-creation meetings and feedback sessions that were used to constantly refine the intervention. During this phase, the group brainstormed, and ideas were discussed and incorporated in the project. Feedback sessions were used to test if the ideas were translated correctly and adjusted where necessary. A total of 78 men contributed to the development of NoMoreC during one or more of the co-creation sessions. Co-creation meetings were held on the development of a hepatitis C prevention toolbox, the online campaign, and outreach activities. The starting point of the meeting about the prevention toolbox was the information collected during the needs assessment. The contents of the NoMoreC toolbox were discussed extensively and decided jointly by the community members and researchers. During the meetings about the campaign and outreach activities, calls-to-action, tone of voice, prevention messages, and different promotion strategies were discussed. Ideas on how to engage men during outreach were proposed and materialized by community members.

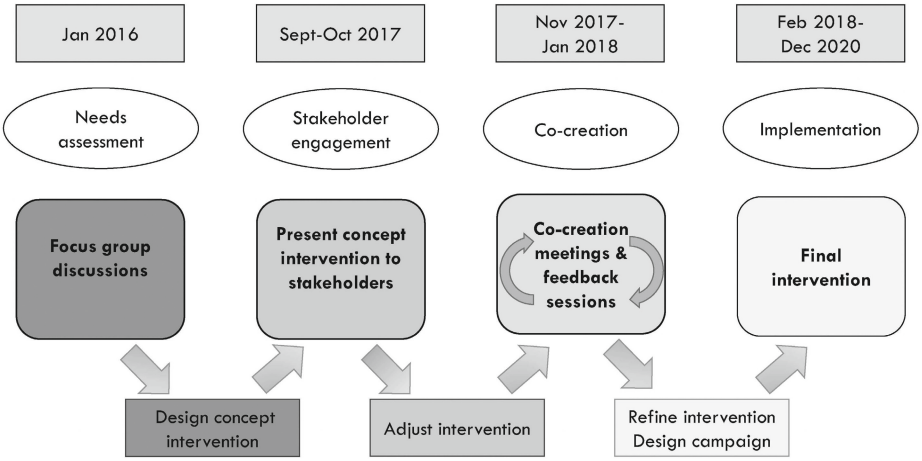

Figure 1. Flow chart of the co-creation process leading to the implementation of the final intervention.

Results

Co-Creation Process

Needs Assessment

The needs and recommendations that were mentioned during the 2 focus group discussions with MSM at risk of HCV are summarized in Table 2.

From the discussions, it became evident that there was a need for clear information about HCV in general and information relevant to the target group and their sexual settings in particular. The participants mentioned that they found it difficult to find reliable information, including information about HCV testing, symptoms, and treatment options. It was mentioned that they felt a good information source was lacking and that a website containing as much information as possible was recommended. They stated that they had a great need for advice regarding their sex lives during the period from the time of infection to cure, including practical tips to limit the risk of transmission during sex. The following quotes illustrate the availability and need for information about HCV.

> *I use google….but the information is really very limited….there is some information, but it is not a lot.*
>
> [Participant who had been HCV-infected]

…I had questions about that (the risk of transmission during sex), but I now realize that I know little about that.

[Participant who had been HCV-infected]

I would want to know about the severity of treatment, the duration of the treatment, whether there are treatment choices, what are the prospects (for controlling HCV), what is the chance of a cure, what side effects does it (the treatment) have, what if it (the HCV infection) becomes chronic?

[Participant who had never been HCV-infected]

….I have had sex with a friend who had HCV. I want to know more about what risk reduction measures you can take to prevent HCV.

[Participant who had never been HCV-infected]

After discussing HCV information needs, the researchers proposed developing a toolbox containing items to assist in HCV risk reduction and asked the participants' opinions. The participants reported being interested in a prevention toolbox if it contained disinfectants that quickly kill HCV, clear explanation on its use, and possibly a list of other products that can assist in risk reduction. Furthermore, 2 other intervention ideas were proposed by the researchers: (1) a home-based hepatitis C testing service and (2) an online checklist to estimate personal risk of contracting HCV. We hypothesized that a home-based testing service could lower barriers to testing, increase testing frequency, and enable MSM to test shortly after potential exposure and thus prevent onward transmission. The rationale of the checklist is two-fold: (1) the proposed checklist would guide men in their decision to test for HCV based on their personal risk, and (2) providing tailored practical risk reduction advice may stimulate risk reduction behavior.

These 2 proposed intervention ideas were received positively and discussed by both focus groups and subsequently added as recommendations (Table 2). Offering easier HCV testing options based on measuring the virus within a short period was considered as important by most focus group members, and it was mentioned that this could assist them in taking control of their own health. Some participants were prepared to pay for such a testing service, while others were not and brought up their concern that men with a low income would be excluded from the service.

Stakeholder Involvement

Based on the needs and recommendations that were brought forward in the focus group discussions, a first concept of the NoMoreC project was designed and

presented to the stakeholders. The project, containing a web-based component, hepatitis C testing service, and face-to-face component, was received with interest. The stakeholder meetings were successful in gathering input and discussing possible support for project implementation. Useful ideas were voiced, and cooperation was offered by various stakeholders: Fetish shop owners offered to distribute or sell potential NoMoreC products as well as assist in project promotion (hand out flyers/posters), and managers of gay sex venues requested to be informed about disinfection on location and made condoms and fisting gloves available for clients, to make their premises "hepatitis C proof."

Co-Creation Phase

During the co-creation phase, different components of the intervention were executed in collaboration with the gay community. These included the development of a prevention toolbox, making content for the project website (eg, explicit comics), filming of testimonials, shooting photos of volunteer models, organization of theme events, and forming a campaign team. The campaign team (the "NoMoreC Boy Scouts") was formed by a group of men at risk of HCV, including men from specific fetish scenes: leather, rubber, and sportswear [28]. All activities were performed on a voluntary basis. For the coordination of the campaign team, a community member was appointed who received a modest fee. The end result of the co-creation phase was the finalized NoMoreC project including web-based and face-to-face components, an anonymous hepatitis C testing service, and a (social) media campaign. The influence and challenges of the co-creation process are mentioned in the following descriptions of each intervention component.

Table 2. Summary results of two focus group discussions determining the needs and recommendations regarding hepatitis C information and testing options.

Theme or topic	Needs	Recommendations
HCV[a] information	Better HCV information sources	Provide good and reliable online information about HCV, provide information specifically relevant to MSM[b], link websites visited by MSM to reliable online information about HCV, provide information about symptoms, and provide an online chatroom where men can ask questions to a professional
HCV risk	Information about which sexual techniques confer a transmission risk and personal advice on HCV risk factors	Provide information about which sexual techniques increase the transmission risk and which sexual techniques are safe as well as a risk checklist

Table 2. Continued

Theme or topic	Needs	Recommendations
HCV prevention	Information about disinfectants effective against HCV and information about sexual behavior that increases HCV risk	Provide information about disinfectants that quickly kill the virus, provide a list with products and how to use these for the prevention of an HCV infection, and create video instructions for risk reduction
HCV testing	Information about different types of tests, information about where you can be tested, and testing possibilities for HIV-negative men outside the general practice	Offer a test that detects the virus (instead of antibodies) that can be used by men who have been infected in the past, offer an HCV test as part of a comprehensive STI[c] screening package at the PHSA[d] STI clinic, communicate test results by a telephone call or face-to-face, and offer a home-testing service to take control over your own health (a convenient option and a good option for MSM outside of Amsterdam)
A positive HCV test result	Information about disease progression, treatment (duration, side effects, regimens, cure rates), consequences of a chronic infection, safe sex while being infected, and partner notification as well as contact with a gay man who has been infected, treated, and cured	Provide information covering the information needs as mentioned under "needs" in the left adjacent column, offer DAA[e] treatment as soon as possible, and offer peer support by bringing a newly HCV diagnosed man in contact with a gay man who has had HCV in the past and was treated with DAAs, to share experiences about being infected and the treatment

[a]HCV: hepatitis C virus; [b]MSM: men who have sex with men; [c]STI: sexually transmitted infection; [d]PHSA: public health service Amsterdam; [e]DAA: direct-acting antiviral.

Web-Based Intervention Components

NoMoreC Website

The project website [29] is available in Dutch and English and targets at-risk MSM (Figure 2). Part of the content was co-created with the community, such as the instructional videos, photos, testimonials, and graphic illustrations. The website was tested by 3 community members. Feedback on their user experience and suggestions for improvement were used to fine-tune the final product. The website was launched in February 2018.

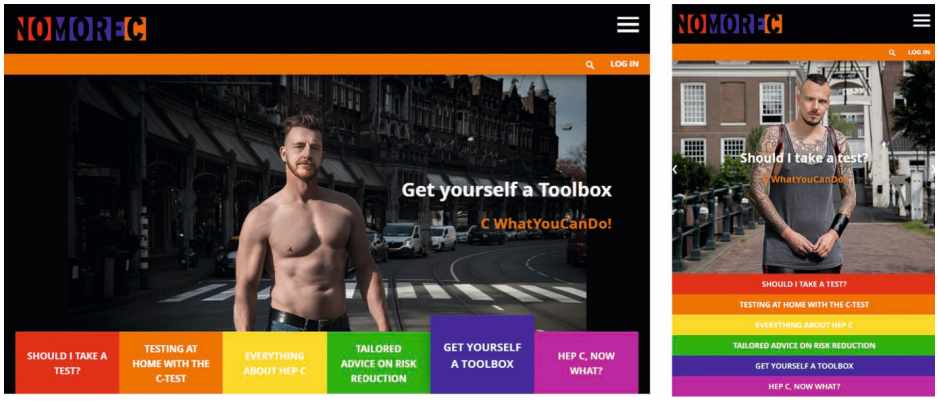

Figure 2. Screenshots of the desktop and mobile versions of the NoMoreC homepage (accessed June 10, 2020).

The website provides information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options, and partner notification. Video testimonials are presented in which men share their personal experiences with hepatitis C, being at risk, and what they do to reduce transmission risk (Figure 3).

Information about HCV transmission routes and risk reduction strategies are illustrated by explicit comic strips of situations familiar to the community (Figure 4). Furthermore, instructional videos can be watched about disinfection to prevent HCV transmission. The website offers personalized risk reduction advice based on sexual practices. Also, personalized test advice is given after answering questions on 6 risk factors, based on a previously validated risk score [30]. An anonymous hepatitis C testing service, which is described in detail in a later section, is incorporated in the website. It offers HCV RNA tests at a reduced cost for €25 per test. A test subscription of 4 tests is offered for €80 to stimulate regular testing.

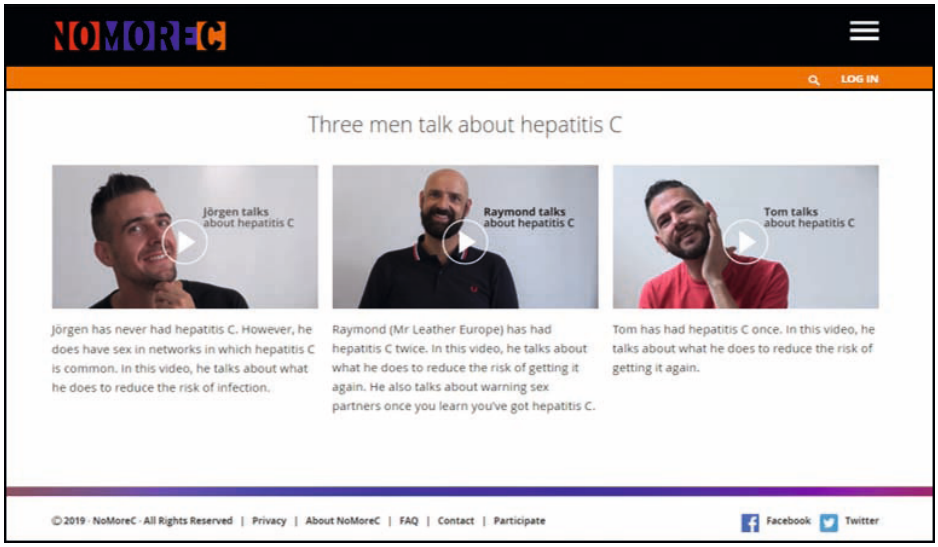

Figure 3. Video testimonials of men from the target population can be watched on the NoMoreC website (accessed June 10, 2020).

Figure 4. Illustration on the website of a risk reduction strategy: cleaning and disinfection of the play area, toys, and yourself before changing sex partners. More information about the products to use is given on the website.

Electronic Learning (e-Learning)
For professionals who see MSM at risk of HCV in their practice, NoMoreC offers an electronic learning (e-learning) package with 3 modules. Module 1 gives information about the NoMoreC project, its target population, and how professionals can use the NoMoreC products in routine clinical practice. Module 2 gives detailed information about HCV risk reduction. Professionals learn about risk factors, tailored risk reduction advice, and behavior change. In the last module, the obligation of health professionals to report hepatitis C to the public health service and partner management is discussed. The e-learning package is accredited by the appointed organizations for nurses and nursing specialists [31,32].

Anonymous Hepatitis C Testing Service
NoMoreC offers an anonymous hepatitis C testing service, using a validated home-based self-sampled dried blood spot (DBS) HCV RNA test [33]. Users of this service are guided through different steps, as depicted in Figure 5, starting with filling out a validated 6-question risk assessment [30]. After receiving test advice, men can purchase the HCV RNA home collection test kit online. Test packages are sent to the chosen address of the potential user (pseudonyms can be used). They are instructed to collect a DBS sample from a finger prick, a procedure that was validated prior to offering the test service [33].Paper instructions for DBS collection are included in the test package, and video instructions are accessible on the website. Users are instructed to send their DBS sample to the laboratory of clinical virology of the Amsterdam University Medical Centers for HCV RNA testing. Test results are communicated within one week via a personal login at the project website. Users who test positive are given guidance on the next step to take, including confirmation of the positive test result at regular health services, access medical treatment and initiate partner notification. A referral letter is provided online to facilitate this follow-up, and a telephone number is given if the user would like to consult a nurse. Post-test information for users who test HCV RNA negative addresses risk reduction strategies and encourages frequent testing. The test service became available in February 2018.

During the development of this testing service, we encountered some challenges. Many community members expressed a strong preference towards receiving a positive test result by telephone call or face-to-face, instead of receiving their test result online. However, the possibility of being able to test anonymously was also seen as important. Therefore, the anonymous testing option was chosen with the possibility for men who test positive to be able to contact a nurse and facilitate linkage to care.

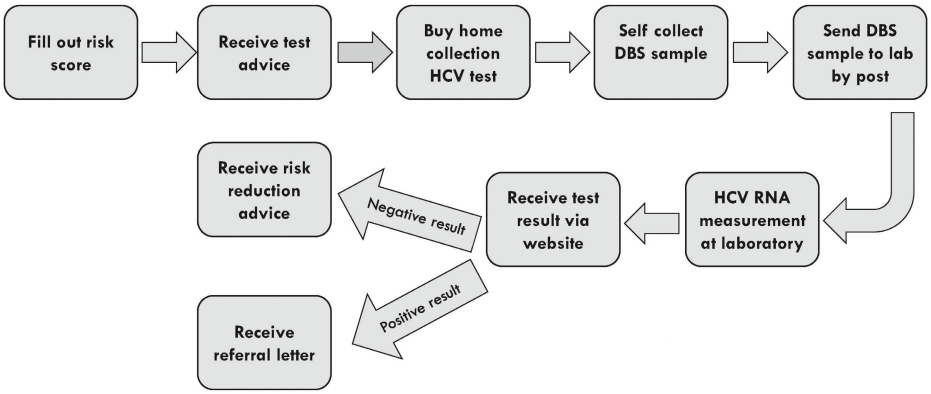

Figure 5. Flow chart of the hepatitis C testing service. DBS: dried blood spot; HCV: hepatitis C virus.

Face-to-Face Intervention Components

Risk Reduction Toolbox

The "NoMoreC Toolbox" contains products to assist in reducing the risk of contracting an HCV infection such as condoms, fisting gloves, safe drug use equipment, and disinfectants (Figure 6). We were advised on the right use and types of disinfectants by hygiene and infection prevention specialists of the National Center for Hygiene and Safety [34]. In addition, the toolbox includes a booklet with practical tips on how to reduce risk of transmission and instructions on the use of the products. Information is also given about testing, treatment, and notifying sex partners.

The toolbox is used by healthcare professionals of the STI clinic and HIV centers in Amsterdam to discuss risk behavior with MSM at risk of HCV and inform them about prevention strategies. The men are offered a box so they can go through it again at home, read the information booklet, and try out the products. The box is also used by the "NoMoreC Boy Scouts" during outreach activities to discuss HCV risk (reduction). Furthermore, the toolbox can be ordered online from the project website or picked up at a gay fetish shop free of charge. The toolbox became available in March 2018.

During the co-creation phase, choices were collectively made on what products the toolbox needed to contain. Consensus was easily reached on the inclusion of the majority of the products except for items for safe drug use. In particular, the suggestion to add needles and syringes to the toolbox led to an extensive discussion. There was disagreement between community members: Some believed that such items would be experienced as shocking while others brought forward that a small group of men inject ("slam") drugs to enhance their sexual experience and that safe drug use items are essential for the prevention of HCV. A compromise was reached

by adding a separate sealed off box to the toolbox, labeled with a sticker explaining that this small cardboard box contains items for safe drug use. After the contents of the toolbox was decided upon, items were ordered, and all boxes were packed by community members. The packing sessions proved to be an effective way to involve men in the project and create a "NoMoreC community."

Figure 6. NoMoreC Toolbox (left) and contents of safe drug use box (right). The NoMoreC Toolbox contains a spray bottle, disinfectant for hands, hydrogen peroxide cleaning wipes, gloves, condoms, a hand washing instruction card, a cardboard safe drug use box and booklet with tips and tricks to reduce hepatitis C transmission risk. The safe drug use box is a small cardboard box that is included in the toolbox. It contains snorting straws, a sharps container, syringes, needles, mixing cups, and alcohol wipes. A sticker with the text "Never share drug use equipment. Pick your own color." is stuck on the box.

Training for Health Professionals

The training developed for health professionals is a 4-hour interactive session about HCV risk behavior, risk reduction measures, and partner management. Prior to the training, participants are asked to complete the e-Learning package. The focus of this training is on improving the participants' communication skills by practicing motivational interview techniques. After the course, a participant is better equipped to ask a client about his risk behavior, give him tailored advice on risk reduction, and support behavioral change.

Tailored Advice to Sex on Premises Venues (SOPV)

In cooperation with infection prevention specialists of PHSA, we advise SOPVs in Amsterdam on HCV prevention and creating an enabling environment for risk reduction for their clients. SOPVs are commercial venues, such as gay clubs and saunas but not brothels, where men can engage in sex. The venues are visited to have a close look at the areas where clients have sex. For each location, tailored advice is given with recommendations for improvement. Points of improvement are given on the use of disinfectants, instructions for cleaning personnel, availability of

gloves, single use anal douches, and safe needle disposal. The recommendations are discussed with the owner or manager of the venue 2 months after the visit, giving them the opportunity to ask for clarification. In addition, a workshop for owners of SOPVs is organized, and support is given to venues when requested.

NoMoreC Campaign

The NoMoreC intervention is promoted with a sex-positive campaign, accepting lifestyle choices of MSM at risk of HCV. The campaign was designed in close cooperation with the community. The goal of the campaign is to raise awareness about the different components of the project that target MSM. With the input of community members, the right words and promotion messages were carefully chosen so that they would resonate with the community. Special care was taken to ensure the messages would neither evoke fear nor stigmatize the target population. The campaign slogan "CWhatYouCanDo!" was chosen to encourage MSM, including men who do not use condoms, to think about which risk reduction strategies they can and are willing to apply to their (sex) lives.

A suite of promotional materials was developed with artwork appealing to the community. It includes posters (Figure 7), flyers, pocket-sized cards, and online banners. Posters hang on waiting room walls of HIV-treatment centers in Amsterdam, the STI clinic of PHSA, and fetish shops. Flyers are handed out to at-risk MSM by health professionals from HIV-treatment centers, the STI clinic, and general practice centers in Amsterdam. Furthermore, at a selection of pharmacies in Amsterdam, flyers are given to men who pick up their HIV medication or pre-exposure prophylaxis. Flyers and pocket-sized cards contain a discount code for the purchase of the NoMoreC HCV RNA test and are also handed out by the "NoMoreC Boy Scouts" during their outreach activities. This campaign team attends gay venues and events to interact with men at risk of HCV. They have one-on-one discussions about hepatitis C, risk reduction strategies, and testing options; demonstrate the use of the Toolbox products; and host quizzes on HCV and risk reduction.

Banner advertisements are shown on gay (fetish) dating and chat apps, including Recon, Scruff, PlantRomeo, and Grindr, prompting men to visit the NoMoreC website, purchase an HCV RNA test, or order a toolbox. The placement of the ads is scheduled around gay events (eg, Gay Pride Amsterdam, Folsom Berlin, The Cruise) to raise awareness about possible HCV transmission risks at these events and focus on the importance of testing. Promotional activities will continue until July 2020.

Figure 7. Promotional poster with the text: How do you reduce your hepatitis C Risk "I always use my own anal douche." The model is a key figure from the target population.

Discussion

The NoMoreC multilevel intervention was created using a co-creation process involving members of the Amsterdam gay community, commercial stakeholders, stakeholders from within the gay community, and health professionals. This process has resulted in the implementation of web-based and face-to-face interventions, including an informative website, an anonymous HCV testing service, a risk reduction Toolbox, a sex positive campaign, a training package for health professionals, and tailored advice to SOPVs. We believe that co-creation has been one of the main strengths of the project, but it also had its challenges and limitations. Co-creation is a time-consuming and intensive process; it took 2 years from the first focus group discussion to the development of the final intervention. At times, exciting ideas could not be materialized, or a compromise had to be reached that was acceptable for

the gay community members, health professionals, and researchers. This required good negotiation and cooperation skills.

To illustrate this, one idea proposed by some members of the gay community was the making of a short film to address hepatitis C risk reduction. In the film, different risk behavior settings and risk reduction strategies would be shown. This would have involved hiring actors and shooting explicit scenes of a group of men at a private sex party, using party drugs, and having sex with multiple partners. There was mixed enthusiasm for this idea. Instead of making a sexually explicit film, it was agreed to make explicit cartoons and instructional disinfection videos (how to disinfect your hands, play area, and sex toys) as an alternative. The cartoonist worked closely with a community member to draw realistic cartoons in a setting recognizable for the target population.

Another challenge was to balance the needs and preferences of the community and data collection needs of the researchers with regard to the HCV testing service. For example, the community members voiced their preference of ordering an HCV test without having to fill out the risk score. However, in order to evaluate the effectiveness of the test service (i.e. test advice, orders placed, and test results in relation to the risk taken), this information is needed. Therefore, the wish of the community to order a test without filling out the risk score could not be granted.

The development of the NoMoreC Toolbox has been a positive experience for both the community and researchers. The co-creation sessions created an atmosphere of co-learning, where the researchers learned more about the context of risk behavior and community members became more knowledgeable about hepatitis C, HCV transmission routes, and risk reduction measures.

The NoMoreC project is ongoing and will run until the end of 2020. We continuously monitor the uptake and reflect on the successes and limitations of the intervention and make adjustments if indicated and possible. By trial and error, we find out how to best reach and involve the target group. The uptake of the intervention at the different levels and the acceptability of the NoMoreC project will be evaluated and reported at the end of the implementation phase.

In conclusion, using the process of co-creation, the multilevel NoMoreC intervention was developed and implemented. The intensive cooperation with the community and stakeholders has allowed us to gather their perspectives and incorporate their ideas in the different components of the intervention. The co-creational approach we have taken may serve as a rich and useful source for others who want to develop culturally and context appropriate HCV interventions.

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Chapter 5

Internet-guided HCV-RNA testing: A promising tool to achieve hepatitis C micro-elimination among men who have sex with men

Tamara Prinsenberg, Janke Schinkel, Paul Zantkuijl, Udi Davidovich, Maria Prins, Marc van der Valk

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Abstract

In the Netherlands, hepatitis C virus (HCV) transmission occurs primarily in men who have sex with men (MSM). By early diagnosis and immediate treatment of acute HCV infections, HCV micro-elimination in MSM is within reach. In cooperation with the community affected, we developed an online HCV RNA home-based self-sampling test service. This service combined online HCV self-risk assessment with the possibility to test anonymously for HCV RNA. The service was available in the Netherlands from February 2018 till December 2020 and was promoted online on various dating sites and offline by community volunteers. Using website user data, test results and an online post-test user survey, we evaluated the service and user experiences. The website page with information about testing was visited by 3401 unique users, of whom 2250 used the HCV-risk assessment tool, 152 individuals purchased 194 HCV RNA tests, and 104 tests were used, of which 101 gave a conclusive result. The target population of MSM at risk was successfully reached with 44.1% of users receiving the advice to test. The test service had a satisfactory uptake (6.8%, 152/2250), a very high HCV RNA positivity rate (10.9%, 11/101) and was considered acceptable and easy to use by most MSM.

We demonstrate that an HCV RNA home-based self-sampling test service is successful in diagnosing HCV infections among MSM. This service could be a valuable addition to existing sexual healthcare services as it may reach men who are otherwise not tested.

Introduction

Since 2000, hepatitis C virus (HCV) outbreaks among men who have sex with men (MSM) living with HIV have been reported globally [1, 2]. The Netherlands has had universal access to direct-acting antivirals (DAA) for HCV treatment in people living with HIV (PLWH) since November 2015. HCV incidence of HIV-infected men who have sex with men (MSM) in the Netherlands has sharply declined after DAA restrictions were lifted in 2015 [3] and HCV-viraemia among MSM living with HIV decreased from 3.9% in 2015 to 0.5% in 2019 [4]. These results suggest that the Netherlands is on track towards HCV micro-elimination in PLWH. Although the HCV reinfection rate also sharply declined from 41.4 per 1000 person-years in 2016 to 11.4 per 1000 person-years in 2019, it remains high [3]. Additionally, HIV-negative MSM using pre-exposure prophylaxis (PrEP) to prevent HIV acquisition are also at substantial risk of HCV infection [5]. A recent meta-analysis estimated a 123-fold higher HCV incidence in HIV-negative MSM using PrEP compared to HIV-negative MSM not using PrEP (pooled HCV incidence of 14.8 per 1000 person-years in HIV-negative MSM using PrEP) [6]. These findings illustrate that additional efforts are needed to achieve HCV elimination goals in MSM.

From the early 2000s, it has become clear that high-risk sexual and drug-related behaviors facilitate the spread of HCV among MSM [7] Early HCV diagnosis and immediate treatment and behavioral risk reduction strategies may be effective to eliminate HCV in MSM [8]. Client-initiated HIV self-testing services have been successful in increasing test uptake among MSM and trans people [9] and may be a promising strategy to expand HCV testing and shorten time between infection and diagnosis.

HCV RNA can be detected in the blood within 7–21 days after infection and is therefore the marker of choice for early diagnosis of HCV infection [10]. In addition, HCV RNA testing can be used to diagnose re-infections.

As part of the NoMoreC project, an innovative, multilevel intervention to reduce the HCV transmission among MSM in Amsterdam, we set up an anonymous Internet-guided HCV RNA self-sampled test service for MSM at risk of HCV infection [11]. The C-test service assists users to assess their HCV-risk, gives personalized testing advice and information about different testing options. The service offers men the possibility to order a self-sampling HCV RNA test kit, send their sample to the laboratory for testing and receive their test result anonymously online. Users who test HCV RNA positive are linked to care to confirm their test result and start DAA treatment.

In this paper, we evaluate the use and outcomes of the test service and report user experiences.

Methods

HCV testing service

The anonymous NoMoreC testing service was available to MSM in the Netherlands and used a validated home-based self-sampled dried blood spot (DBS) HCV RNA test [12]. The testing service was part of the NoMoreC project, a multilevel intervention developed and implemented at individual, community, healthcare professional, context, patient and network level. The co-creation process and development of the HCV testing service have been previously described in detail [11]. Briefly, we conducted focus group discussions with a group of MSM at risk of HCV to identify the needs regarding hepatitis C information and testing options. The community group recommended to use a test that detects the virus (instead of antibodies), also in men who have been infected in the past, and to offer a home-testing service. Based on this recommendation, an anonymous home-based HCV RNA testing service guided by personal testing advice was proposed, which was received positively by the focus group participants. Subsequently, a website was developed (www.NoMoreC.nl) targeted at MSM at risk, providing information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options, and partner notification. The website, available in Dutch and English, offered personalized online test advice, and the possibility to anonymously purchase an HCV RNA test, called the C-test. Test advice was given, based on the validated HCV-MOSAIC score which consist of 6 self-reported risk factors (i.e. condomless receptive anal sex in previous 6 months, sharing of sex toys in previous 6 months, fisting without gloves in previous 6 months, injecting drugs in previous 12 months, sharing straws when snoring drugs in previous 12 months, and self-reported ulcerative STI (syphilis, genital herpes or lymphogranuloma venereum infection) in the previous 12 months) [13]. The HCV-MOSAIC score was developed to identify HIV-positive MSM at high risk for an acute HCV infection. The performance of the score among HIV-infected MSM in the development study showed a sensitivity of 78.0% and a specificity of 78.6% for acute HCV.13 The score was validated, using data from three studies, showing a sensitivity ranging from 73.1% to 100% and specificity from 56.2% to 65.2% [13].

Users of the C-test service at risk of infection according to the HCV-MOSAIC risk score, and those who had been notified for HCV by a sexual partner, received the advice to get tested for HCV. Regardless of the test advice, users could subsequently purchase a test kit online (€25/test or €80/4 tests). Discount codes of 50%–100% could be used by those users who had seen the NoMoreC online promotional activities or flyers. Test kits were sent to the given address of the potential users

(pseudonyms could be used), containing a DBS card with a unique number and barcode. Names or other identifying information were not printed on the DBS card and therefore could not be linked to the test result ensuring the anonymity of the service users. Users were informed by paper illustrations [14] and an online instructional video [15] on how to collect finger-prick blood samples. They were instructed to prick their finger with the lancets provided, place a drop of blood in each of the five circles on the DBS card, air dry the sample, place the DBS card in a grip seal bag with desiccant and subsequently in an envelope box. The envelope box needed to be placed in a postage-paid return envelope addressed to the laboratory of clinical virology of the Amsterdam University Medical Centers for HCV RNA testing. All packaging materials were provided in the test kit. Laboratory staff used the DBS card number and barcode for processing the test and authorizing the result. Users received an automated email notification when the result was entered in the system, which they could subsequently access with their personal login to the project website. Positive test results included a link to a referral letter, which users were advised to use for confirmation of their test result at the STI clinic, HIV treatment center or GP practice. In addition, they were advised to prepare a contact list of all their sexual partners in the last 6 months to facilitate partner notification. Furthermore, a telephone number of a nurse practitioner was given to facilitate follow-up and give guidance to the user. For users who tested HCV RNA negative, post-test information addressed risk reduction strategies. In case of an inconclusive result, users were given the possibility to order a new test kit free of charge.

In February 2018, the NoMoreC website was launched and test kits could be purchased. The sale of test kits continued until November 2020. DBS samples, of the sold test kits, could be used until December 10, 2020. Online test results were accessible for the user until February 2021.

HCV RNA testing

Upon receipt of the DBS sample in the laboratory, two spots were cut out of the DBS card and eluted in L-6 buffer (500 g GuSCN in 91.7 mL 0.2 M EDTA [pH 8.0], 10.12 mL Triton X-100 and 416.7 mL 0.1 M Tris–HCl [pH 6.4]). The CAP/CTM assay (COBAS Ampliprep/COBAS TaqMan; Roche Diagnostics) was used for extraction, amplification and quantification of HCV RNA on DBS eluates.

Campaign design

From March 2018 till November 2020, the C-test service was promoted online on various gay dating applications and offline by the NoMoreC boy scouts, a team of volunteers recruited from the local community engaged in the project. Members of the team visited different gay events to promote the testing service. In addition, a promotional flyer was distributed by a leading pharmacy in Amsterdam (DC apotheek Valeriusplein) specialized in providing prescribed PrEP medication to

men throughout the Netherlands. Discount codes of 50%–100% for a single test were distributed through the promotional activities to motivate men to purchase the C-test.

Post-test survey

All users, who ordered a C-test kit, received a link to an online questionnaire by e-mail, 3 weeks after placing the order. No reminders to fill in the questionnaire were sent. The questionnaire, available in Dutch and English, contained questions about and reasons for using the C-test service, acceptability of the C-test service, satisfaction with the service and NoMoreC website and possible problems with home sampling. Answer options included 5-point Likert scales, lists with pre-defined answers and open text fields (see Appendix 1 for the questionnaire and answer options). Participants gave their informed consent for scientific use of the questionnaire data by clicking the consent box prior to initiating the online questionnaire. The questionnaire responses were not linked to test results or users email addresses to ensure anonymity.

Measures and outcomes

Website user data and test results were exported from the website, for the period 1 February 2018 to 31 December 2020. We collected data on:

- Total number of visitors to the NoMoreC website;
- Total number of men who requested online test advice;
- Total number of men who received the advice to get tested, based on the HCV-MOSAIC score;
- Total number of men who received the advice that testing is not necessary, based on the HCV-MOSAIC score;
- Total number of men who received the advice to test based on a partner notification;
- Total number of men who ordered a C-test kit;
- Total number of men who used a discount code when ordering the kit;
- Total number of men who collected a DBS sample and returned it to the laboratory;
- Total number of positive and negative test results.

Descriptive analyses were used to assess the uptake (proportion of users who ordered a test kit after requesting test advice) return rate (proportion of ordered tests that were returned), positivity rate (proportion of test results that were HCV RNA positive) of the test service and the characteristics of the users who ordered a test kit.

Furthermore, we assessed the level of agreement with statements regarding usability, acceptability and satisfaction of the service among all questionnaire respondents.

Results

Uptake and results testing service

Between 1 February 2018 and 31 December 2020, the NoMoreC website was visited by 43,075 unique users (Figure 1). A third of the website users were returning visitors. The website page with information about testing was viewed 7127 times, of which 3, 401 were unique page views (i.e. number of sessions in which the specified page was viewed at least once by a unique user). Of the users who visited the webpage about testing 66.2% (2250/3401) requested online personalized test advice (Figure 1). A total of 194 HCV RNA tests were ordered by 152 men, resulting in an overall uptake of the test service of 6.8% (152/2250). Discount codes were used by 73 men (48.0%) and 79 men (52.0%) paid the regular price of €25 for one test. Of the purchased tests, 53.6% (104/194) were returned to the laboratory, of which 97.1% (101/104) gave a conclusive result. Eleven out of 101 (10.9%) were HCV RNA positive.

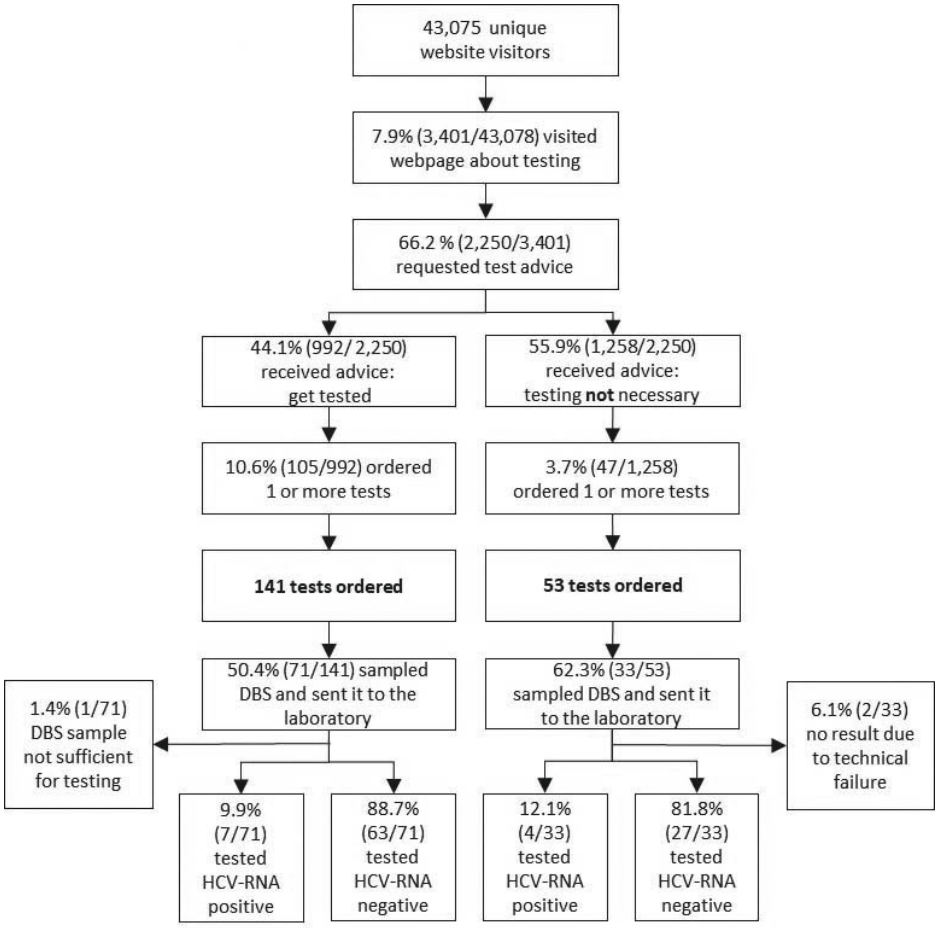

Figure 1. Uptake and results of the Internet-guided HCV RNA testing service from 1 February 2018 to 31 December 2020. DBS, dried blood spot; HCV, hepatitis C virus

Of the website users requesting test advice, 44.1% (992/2250) received the advice to get tested, based on the risk score or having been notified by a sex partner, and 55.9% (1258/2250) received the advice that HCV testing was not necessary. Of the 992 website users who received the advice to test, 105 men (10.6%) ordered 141 test kits, of which 71 (50.4%) were returned to the laboratory. Seven out of 71 tests (9.9%) were HCV RNA positive, 63/71 (88.7%) were negative and 1/71 (1.4%) could not be tested because the user had not sampled a sufficient amount of blood. Of the 1258 website users who received the advice that testing was not necessary, 47 (3.7%) ordered 53 test kits, of which 33 (62.3%) were returned to the laboratory. Four out of 33 tests (12.1%) were HCV RNA positive, 27 (81.8%) negative and 2 (6.1%) were inconclusive because of a technical failure.

Among the users who received the advice to test, 16.2% (161/992) were notified by a sex partner, of whom 11 (6.8%) received the advice based on the partner notification only and 150 (93.2%) also based on the risk score. Nine per cent of the notified men (15/161) ordered one test. All 15 tests were returned to the laboratory of which two tests (13.3%) were HCV RNA positive.

Characteristics of survey participants

A total of 152 men who had ordered one or more tests received an email with a link to the online questionnaire between 22 February 2018 and 31 December 2020. The questionnaire was started by 86 participants and completed by 54, resulting in a response rate of 35.5% (54/152). All participants (n = 54) were MSM living in the Netherlands. The majority (44/54, 81.5%) were born in the Netherlands. The median age was 46 years (IQR 39–53). Two thirds reported they were HIV-negative (35/54, 64.8%) of whom 54.3% (19/35) were using PrEP. An HIV-positive status was reported by 15/54 men (27.8%), and 4/54 (7.4%) did not disclose their HIV status. Two thirds reported they had previously been tested for HCV (35/54, 64.8%). Previous testing took place at the STI clinic (17/35, 48.6%), hospital (12/35, 34.3%), GP practice (10/35, 28.6%) and NoMoreC testing service (3/35, 8.6%). Seven participants reported they had been tested at more than one location.

Usability, acceptability and satisfaction with the test service

Most survey respondents (44/54 81.5%) had used the test kit, of whom 3/44 (5.6%) had reported a positive test result, 27/44 (61.4%) reported a negative test result, 11/44 (25.0%) did not disclose their result and 3/44 (6.8%) had not yet received their test result. The three users who received a positive test result had their test result confirmed at their GP (n = 1) or STD clinic (n = 2) and were all linked to care. Ten respondents had not (yet) used the test kit for the following reasons: I am waiting until I have a reason to test (4/10, 40.0%), I have not had time to do the test yet (1/10, 10.0%), I was recently tested for HCV (1/10, 10.0%) and 4/10 (40.0%) respondents did not give a reason.

The majority of respondents were positive regarding C-test service usability, acceptability and satisfaction (Figure 2). Half of the respondents (27/54) reported that they found it easy to self-sample, 31.5% (17/54) found it difficult and 18.5% (10/54) did not answer the question. The main problem with sampling blood was to collect enough blood from the pricked finger to fill five circles on the sample card, which was reported by 16 of the 17 respondents who indicated self-sampling was difficult.

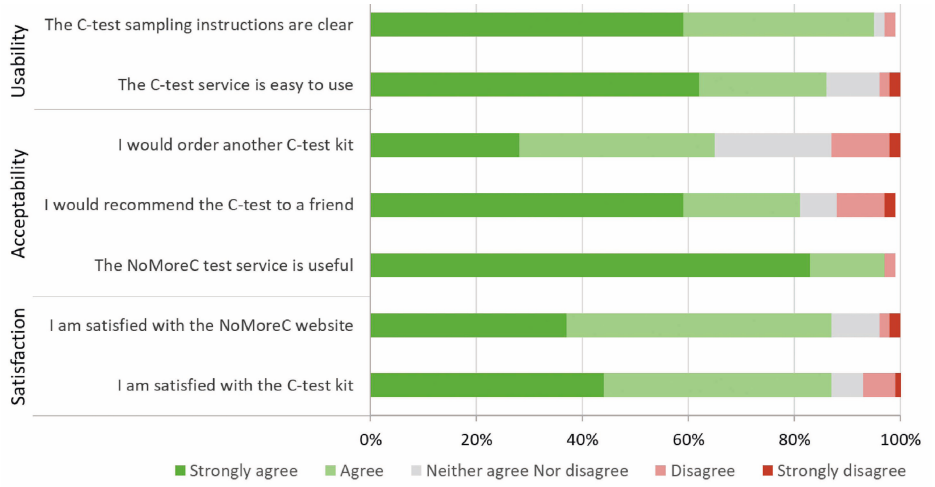

Figure 2. Level of agreement with statements about C-test usability, acceptability and satisfaction of 54 survey respondents who had ordered a C-test

Table 1. Reasons for use of the C-test service and suggestions for improvement reported by 54 participants who completed a survey 3 weeks after ordering a C-test kit. More than one reason for C-test service use could be given. Suggestions for improvement were given by 8/54 participants.

Reasons for using the C-test service
· I want to make sure I am not infected with HCV (n=33, 61.1%)
· I was worried I may be infected with HCV (n=20, 37.0%)
· It saves time (n=14, 25.9%)
· The test is cheap (n=14, 25.9%)
· I wanted to buy a test kit anonymously (n=10, 18.5%)
· I wanted to have a test-kit at home in case I need one in the future (n=8, 14.8%)
· I was curious about how the test service worked (n=7,13.0%)
· I prefer not to talk about HCV-testing with my GP (n=6, 11.1%)
· I do not know any other way of getting tested for HCV (n=5, 9.3%)
· I bought the test kit for someone else (n=2, 3.7%)
· Because I take PrEP, I need to get tested for HCV (n=2, 3.7%)
· I was notified by a sex partner (n=1, 1.9%)
Suggestions for service improvement
· Improve the self-sampling instruction and give tips on blood collection (n=3, 37.5%)
· Improve instruction regarding packaging and posting of the blood sample (n=2, 25.0%)
· Integrate the C-test service with an existing online STI testing service (n=2, 25.0%)
· Include more than two lancets in the test kit (n=1, 12.5%)

Reasons for use of test service

Reasons for using the C-test service were reported by all questionnaire respondents (n = 54), and suggestions for improvement of the C-test were given by 8/54 respondents. The most common reasons for using the service were as follows:

confirming their HCV-negative status, having concerns about being HCV-infected, saving time and the cheap price of the test (Table 1).

Discussion

To our knowledge, this is the first time an Internet-guided anonymous home-based self-sampling HCV RNA test service, targeted at MSM at risk of acquiring HCV, was launched and evaluated. The service was developed and implemented in close conjunction with the community affected and combined a personalized test advice with the possibility to test anonymously for HCV [11]. We demonstrate that this service was successful in reaching its target population of MSM at risk of HCV as 44.1% of the users received the advice to test after filling in a previously validated questionnaire [13], indicating that these men had been at risk of HCV in the past 6–12 months. Furthermore, we demonstrated that the service successfully diagnosed HCV infections with a positive test result in 10.9% of tests performed.

Considering that our C-test service was a user-initiated paid service offered in a country where STI testing is free of charge for MSM, the uptake of testing of 6.8% was satisfactory. Testing uptake following the advice to test might be higher as some men could have chosen to be tested elsewhere (e.g. at a STI clinic or HIV treatment center). Interestingly, 3.7% of men who were advised that testing was not necessary, purchased a test kit. This suggests there is a need for men to know their current HCV status and that our service promoted pro-active testing. This is in line with our finding that 15% of the online questionnaire respondents reported as reason for purchasing the test its future use.

To the best of our knowledge, there are no other studies describing online user-initiated, home-sampled HCV RNA testing services, and its uptake. Others have reported the uptake of home-sampled HIV and/or STI testing services for MSM. A Dutch pilot program offering home-sampling STI testing (chlamydia, gonorrhea, hepatitis B and syphilis) to MSM living with HIV reported an uptake of 58% among their target group [16]. The pilot was implemented in an HIV treatment center, where healthcare providers offered free STI sampling kits to their clients during routine HIV care visits. The healthcare provider-initiated nature of the service as well as being free of charge may explain the higher uptake. A study from the UK reported an uptake of free home-based testing for HIV and/or hepatitis B and syphilis of only 1% among MSM [17]. Similar to our C-test service, this service was user-initiated and offered online. The higher uptake among MSM in our study may be explained by the increased HCV risk awareness and testing needs of the group of MSM who were reached by our campaign and visited the NoMoreC website. A study describing a risk-based HCV antibody testing intervention targeted at people at risk in the

general population in the Netherlands, reported an uptake of 28% among those who received the advice to test [18]. This study also used an online questionnaire to assess risk and advice testing, but unlike our service participants received reminders by email or SMS and were referred to a laboratory for testing. This could indicate that sending reminders and offering the option to have blood sampled at a laboratory as an alternative to self-sampling at home, may increase uptake.

We found that our C-test service scored high on measures of usability, acceptability and satisfaction among its users. The main reasons for MSM to test for HCV through our service were to make sure they were not infected with HCV and being worried about an HCV infection. Time-saving, the relatively low price and the anonymous character of the service were also indicated as important. These factors are important in facilitating access to HCV testing. Some users stated that they did not know another way of getting tested for HCV, which shows that the C-test service has made testing more accessible. Hence, our service is a valuable addition to HCV testing at sexual health clinics, HIV treatment centers and GP practices.

Self-sampling of a good-quality DBS is crucial to the success of the service and dependent on clear sampling instructions. In our study, almost all users were able to sample an adequate DBS-sample and rated the instructions as clear or very clear. Yet, suggestions for improvement of the self-sampling instructions were given by the users. For future programs that will use a similar home-sampled testing approach, we recommend the production of easy-to-follow step-by-step instructions, including sampling, drying, packaging and posting instructions of the sample.

The overall rate of self-sampling and returning the home-based testing kits was 53.6% (104/194), which falls within the range of home sampling return rates for HIV and STI testing found in other studies (43.8%–84.5%) [16, 17, 19, 20]. The return rate could potentially have been higher if users had received reminders, as shown by studies that provided of self-sampling STI tests [16]. Extending the evaluation period may also have resulted in a higher return rate as some users had indicated to have bought the kit for future use. These users may not have been at risk during the study period and hence had not returned their test.

Eleven new HCV infections were identified among the 104 MSM who returned their sample in the project period of 3 years (2018–2020). It is likely that these infections are among the 142 notified acute HCV infections among the general Dutch population, in 2018 and 2019 [21], as all HCV RNA-positive participants of the post-test survey reported they had followed the advice to confirm their test result at regular healthcare services.

The C-test service yielded an overall positivity rate of 10.9%. Among users who had received the advice to test based on the risk score or partner notification, the positivity rate was 9.9%. These rates are remarkably high, especially compared to a recent study at the STI clinic in Amsterdam that found a HCV RNA positivity rate of 1.2% among MSM with HIV and transgender women who also were advised to test according to the HCV-MOSAIC score [22]. Compared to free self-sampling HIV services in high-income countries for MSM, which have yielded positivity rates between 0.3% and 6.1% [23], the positivity rate of our testing service is also high.

We recommend the continuation of an Internet-guided anonymous home-based HCV RNA self-sampling testing service for MSM at risk of HCV, with targeted campaigns to encourage testing. Our service can facilitate early HCV diagnosis and prompt treatment, if the target population use the service to test shortly after having been at risk. MSM at increased risk of HCV are recommended to test every 3–6 months and to treat a recently acquired HCV infection immediately after diagnosis with DAAs [24]. Regular testing and high treatment success rates greatly reduce the HCV community reservoir and limit the pool for onward transmission [25]. A recent Dutch modelling study among HIV-positive MSM showed that early DAA treatment for acute HCV-infected men is a cost-saving prevention approach which indeed reduces the HCV incidence among this target population [26]. Assessing a reduction in time between infection and diagnosis when compared to standard care was outside the scope of our evaluation. However, this would be of interest to determine in the future as to be able to evaluate the impact of the testing intervention on the HCV epidemic.

This study also has limitations. First, we could not assess the reasons why men at risk did not order a test or if they decided to test elsewhere following a test advice. It would be insightful to understand their test behavior and barriers to use of the C-test service, and to evaluate whether they can be lowered to increase uptake. Second, less than a third of the users of the test service completed the online questionnaire. Users who did respond may have been more positive or negative about the service than those who did not. Nevertheless, the questionnaire data were valuable to give an understanding of the user experiences. Third, we have limited knowledge about the reasons for not using the test kit for those men who did buy a kit but did not return their home-sampled DBS.

In conclusion, the C-test service is a practical solution to improve access to HCV testing for MSM at risk and was considered acceptable and easy to use by most MSM. Our approach of offering online test advice combined with an anonymous Internet-guided HCV RNA home-based self-sampling test service, in a country with unrestricted access to DAAs, contributes to reaching micro-elimination of hepatitis C among MSM. If the C-test service could be integrated in an existing Internet-guided

STI testing platform, it could further increase access to testing and improve testing convenience for MSM at risk of HCV. Alternatively, the service could be offered as an additional testing service for those at risk of HCV infection next to routine HCV testing during PrEP and HIV care.

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Conflict of interest

The authors have no conflicts of interest to declare

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Supporting information

Questionnaire sent to toolbox recipients in Dutch and English

Introduction
We believe it's important that the C-test service of NoMoreC meets your needs. Below you'll find our feedback questionnaire. In it, we ask what you think of the NoMorec website & testing service, and your reason(s) for using it. It'll only take a few minutes, and we'll be grateful for your help!

Questions:
First, we ask for your permission to analyze your answers to the questions below. All data is treated with complete confidentiality and information is never linked to your name or (e-mail) address. You can read more about our research at www.NoMoreC.nl.

O I agree

1. **Why did you order a C-test through www.NoMoreC.nl?**
   You may choose more than one answer.
   - o I was worried that I might have hepatitis C
   - o I wanted to be sure that I didn't have hepatitis C
   - o I wanted to buy a test kit anonymously
   - o It was quicker to order it online
   - o It was cheap
   - o I didn't want to have to talk to my GP about taking a hepatitis C test
   - o I don't have a GP
   - o I don't know of any other way of taking a hepatitis C test
   - o I was curious about how the test worked
   - o I wanted a test kit in case I need one sometime later
   - o I bought the test kit for someone else
   - o Other reason, namely ….
2. **Have you used the test kit yet?**
   - o Yes
   - o No

2 a. When answer to question is "No": Why haven't you used it yet?

- o I haven't had time yet
- o I'm waiting until I have a reason to test myself
- o I haven't received the test kit yet
- o The test kit arrived with parts missing/The package arrived open
- o I gave the test kit away
- o Other reason, namely ….

3. **Would you order another test kit from NoMoreC?**
    - o Definitely
    - o Probably
    - o Maybe
    - o Probably not
    - o Definitely not
4. **Would you recommend the test kit to a friend?**
    - o Definitely
    - o Probably
    - o Maybe
    - o Probably not
    - o Definitely not
5. **Are you HIV-positive or -negative?**
    - o HIV-positive (I have HIV)
    - o HIV-negative (I do not have HIV); I was tested less than 6 months ago
    - o I don't know; my last HIV test was more than 6 months ago
    - o I don't know; I have never been tested for HIV?
    - o I do not want to disclose my HIV-status

5 a. When answer Q.5 is "HIV-negative" or "I don't know":
Do you take PrEP (Pre-Exposure Prophylaxis)?

- o Yes
- o No.

6. **Have you ever been tested for hepatitis C? Don't count your recent test via www.NoMoreC.nl.**
    - o Yes
    - o No
    - o I don't know

6 a. When answer is "Yes": Where did you take the test?
You may choose more than one answer.

- o GP's office
- o GP's office, in the context of my PrEP use
- o GGD Amsterdam (STI clinic)
- o GGD Amsterdam, in the context of my PrEP use
- o At a hospital
- o At home, with a home-sampling kit via www.NoMoreC.nl
- o At home, with a self-test kit that gave me the results directly
- o At a laboratory
- o Somewhere else, namely .....

7. **Generally speaking, how satisfied are you with the test kit from www.NoMoreC.nl?**
    - o Very satisfied
    - o Fairly satisfied
    - o Neither satisfied nor dissatisfied
    - o Fairly dissatisfied
    - o Very dissatisfied

When you have used the test kit please continue with **question 8**
When you have not used the test kit please continue with **question 12**

8. **Which instructions did you follow for taking a blood sample? (You may choose more than one answer.)**
    - o The instructions that came with the test kit
    - o The instructional video on www.NoMoreC.nl
    - o Other, namely …
9. **How clear were the instructions for taking a blood sample?**
    - o Very clear
    - o Fairly clear
    - o Neither clear nor unclear
    - o Fairly unclear
    - o Very unclear
    - o I didn't use any instructions.
10. **Did you have any problems taking a blood sample?**
    - o Yes
    - o No.

10 a. When answer is "Yes": What problems did you have?

11. **What did your test result say (when you checked on www.NoMoreC.nl)?**
    - o Positive (That I had hepatitis C)
    - o Negative (That I did not have hepatitis C)
    - o Test unsuccessful
    - o Skip this question

11 a Q.11= positive: Did you have yourself tested again to confirm the results?
    - o Yes, by my GP
    - o Yes, at a hospital
    - o Yes, at GGD Amsterdam's STI clinic
    - o Yes, somewhere else, namely ……
    - o No.

11 b Q.11= positive: Did you use NoMoreC's referral letter?
    - o Yes
    - o No.

12. Do you agree or disagree with the following statement: "I find the NoMoreC testing service easy to use".
- o Strongly agree
- o Agree
- o Neither agree nor disagree
- o Disagree
- o Strongly disagree

13. Do you agree or disagree with the following statement: "I find the NoMoreC testing service useful".
- o Strongly agree
- o Agree
- o Neither agree nor disagree
- o Disagree
- o Strongly disagree

14. Do you have any suggestions for improving the test service?

Chapter 6

Usability, acceptability, and self-reported impact of an innovative hepatitis C risk reduction intervention for men who have sex with men: A mixed methods study

Tamara Prinsenberg, Joël Illidge, Paul Zantkuijl, Maarten Bedert , Maria Prins, Marc van der Valk, Udi Davidovich

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Abstract

Hepatitis C virus (HCV) elimination among men who have sex with men (MSM) is unlikely to be feasible without effective behavioural interventions. We developed a multilevel intervention to reduce HCV transmission among MSM in Amsterdam. The intervention includes a toolbox to facilitate risk reduction among MSM and support healthcare professionals in risk reduction counselling. To assess the use of the toolbox and its impact on behavior, we conducted a mixed-methods study. We collected data through online questionnaires (n = 49), and in-depth interviews with MSM at risk of HCV (n = 15) and healthcare professionals (n = 7). We found that the toolbox has been well received by MSM, increased awareness of HCV risks and has facilitated preventive behaviours and risk reduction communication with peers. Professionals reported the toolbox to be a useful aid for discussions about HCV risk and risk reduction strategies with their clients.

Introduction

Since 2000, hepatitis C virus (HCV) outbreaks among HIV-positive men who have sex with men (MSM) have been reported globally [1]. In the Netherlands, a decline in primary HCV infection and HCV reinfection has been observed in this population after the introduction of unrestricted access to direct-acting antivirals (DAAs) in 2015 [2]. Between 2008 and 2015 the HCV incidence fluctuated between 8.7 and 13.0 per 1000 person-years and significantly declined to 6.1 per 1000 person years in 2016. Between 2017 and 2019, HCV incidence fluctuated between 4.1 and 4.9 per 1000 person-years. HCV reinfection incidence also declined from 41.4 per 1000 person-years in 2016 to 11.4 per 1000 person-years in 2019, but it remained high, illustrating the ongoing HCV transmission in HIV-positive MSM in the Netherlands [2].

HCV incidence among HIV-negative MSM is more challenging to predict as HIV-negative MSM are normally not in routine clinical care. Two systematic reviews showed a 16-to-19-fold lower HCV incidence in HIV-negative MSM compared to HIV-positive MSM including studies from 2000 to 2016 (0,4/1000 person years in HIV- negative and 6.4–7.8/1000 person years in HIV-positive MSM) [3, 4]. However, the introduction and increased uptake of pre-exposure prophylaxis (PrEP) against HIV infection, has resulted in changed patterns of sexual networks and behaviour among HIV-negative MSM [5, 6]. A recent meta-analysis, including studies from 2000 to 2019, estimated a 123-fold higher HCV incidence in HIV-negative MSM using PrEP compared to HIV-negative MSM not using PrEP and a pooled HCV incidence of 14.8 per 1000 person years in HIV-negative MSM using PrEP [7]. An additional concern is the high HCV reinfection incidence among HIV-negative MSM using PrEP. During follow-up in a PrEP demonstration project in the Netherlands an HCV re-infection incidence of 278 per 1000 person years was found [6].

HCV transmission among MSM occurs mainly through sexual contact [8]. Several studies have identified certain sexual techniques and settings as risk factors for HCV infection, including condomless anal intercourse, (unprotected) fisting, sharing of sex toys, group sex and chemsex (the use of recreational drugs immediately before and/or during sex to facilitate or enhance sexual pleasure) [8, 9]. In addition, having an ulcerative sexually transmitted infection (STI), injecting drug use, sharing of straws or other equipment for snorting drugs, sharing anal douching equipment and rectal bleeding are also associated with an increased risk of incident HCV infection [8, 9]. A study in the Netherlands found that receptive condomless anal intercourse, sharing of sex toys, group sex, anal rinsing before sex, having 10 or more sex partners in the last 6 months are strongly associated with HCV reinfection [10]. To prevent HCV (re)infections among MSM, scaling up HCV treatment and the implementation of risk reduction interventions for MSM are recommended [11]. Evidence on how to achieve HCV behavioural risk reduction in MSM is limited. Up

to now, the focus of sexual risk reduction interventions has been on the use of condoms to prevent the transmission of HIV and other STIs. Yet, the prevention of HCV requires more than promoting condom use alone. Informing men at risk about the possible HCV risk factors other than anal intercourse, as well as motivating them to integrate such risk reduction strategies into their sex lives are essential. The first HCV specific sexual risk reduction intervention was developed in 2016 as part of the Swiss HCVree trial [12]: an intervention for HIV/HCV co-infected MSM provided in combination with DAA treatment and consisting of individual counselling sessions aimed at reducing sexual risk taking [13]. In 2017, we initiated the NoMoreC project, a multilevel intervention aimed at reducing HCV transmission among all MSM at risk of HCV in Amsterdam [14]. Our approach was to target MSM based on their sexual practices, rather than on their HIV or HCV-status. NoMoreC includes web-based and face-to-face components as well as an anonymous testing service. The NoMoreC website (www.nomorec.nl) provides information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options, and partner notification. The face-to-face component comprises a risk reduction toolbox, training for health professionals, and providing tailored advice to sex on premises venues. NoMoreC is promoted by a voluntary community campaign team.

In this study we focus on one component of the project, the NoMoreC toolbox. The approach used to develop the toolbox was innovative, and such approach was not applied and evaluated elsewhere before. The toolbox development was based on the information motivation and behavioural skills (IMB) model [15], as IMB-based interventions have shown to be effective in preventing risky sexual behaviours among risk groups [16–18]. The NoMoreC toolbox contains practical tools, to encourage HCV risk reduction in different settings (e.g. during one-on-one sex, group sex, sex parties, sexualized drug use). Examples of these tools are condoms for safer sex, gloves and hand disinfectant for safer fisting, single use drug equipment for safer chemsex and various cleaning and disinfection products to create safer conditions for group sex.

The Toolbox is the first comprehensive HCV risk reduction tool to provide information on HCV risks in combination with a broad range of products for risk reduction and, furthermore, it has been co-created with the gay community. We conducted a mixed-methods study among MSM at risk of HCV and healthcare professionals to assess the use of the toolbox and its impact on behaviour. We aimed to identify reasons for obtaining the toolbox, motives and barriers to its use, measured aspects of usability and acceptability, and assessed the toolbox's impact on HCV awareness and personal behaviour change, as self-reported by toolbox users.

Methods

Toolbox development and distribution

The development of the toolbox was guided by a co-creation process that included various sessions with a group of MSM. This group was formed specifically for the NoMoreC project and was composed of members of the Amsterdam gay community. It included men at risk of HCV, men who had been HCV-infected in the past, and men who had concerns about becoming infected. The IMB model [15] applied by us to the HCV context, suggests that individuals will initiate and maintain HCV preventive behaviours if they are well-informed regarding HCV, HCV transmission routes, HCV infection risks, and possible preventive behaviours, are motivated to prevent infection, and perceive themselves as being capable of applying the recommended preventive strategies. In our first meetings with the community group we explored the needs and views of participants regarding a practical, action-based approach to HCV risk reduction. Based on the first feedback round we set the following IMB-inspired goals: on the information and motivation levels we wished to provide detailed HCV risk information and information on practical risk reduction steps to avoid HCV transmission and increase the response efficacy (i.e. increase a person's belief that the recommended steps will be effective in avoiding an HCV infection) and hence improve attitudes towards preventive behaviours. On the skills level, we wished to provide (illustrated) tips on how to perform such steps to increase perception of self-efficacy an provide the actual products that can be used to perform the suggested steps. We subsequently held several sessions about the actual toolbox content and supporting instructional materials. During these sessions it became clear that many community members were uncertain about how to effectively clean and disinfect their toys, hands and other surfaces, and they voiced their need for cleaning and disinfection instructions. This resulted in adding a number of cleaning and disinfection materials and instructions to the toolbox. An example of these instructions is the step-by-step explanation on how to disinfect hand and forearms: 1) wash with soap to remove all lube from your skin and rinse well; 2) dry your skin, 3) make a cup with one hand and fill it with hand disinfectant; 4) disinfect hands and forearms by rubbing hand disinfectant for at least 30 minutes. Another example is how to disinfect sex toys and anal douches which was explained in 4 steps: 1) Clean sex toys and anal douche with dish-washing liquid to remove all lube and rinse well; 2) mix 1 part bleach with 9 parts cold water; 3) submerge for 5 minutes in the bleach solution: 4) rinse thoroughly with water and wipe dry with a clean towel.

Decisions on the addition of other products to the toolbox were collectively made with the community (Fig 1). Consensus was easily reached for most of the products except for the safe drug use equipment. The suggestion to add needles and syringes to the toolbox led to an extensive discussion within the community group. Some members were in favor of including safe drug use equipment as drug use is a reality

in their environment. Whilst other members believed that injection equipment would be experienced as shocking or may trigger drug use. However, because safe drug use is essential to prevent HCV among men who engage in chemsex, a compromise was reached and the safe drug-use equipment was included in the toolbox but packaged in a separate sealed off box, so that the injection equipment was not directly visible when opening the toolbox. After the contents of the toolbox was decided upon, items were ordered, and all boxes were assembled and packed by the community members. A total of 700 toolboxes were packed; 450 in 2018 and an additional 250 in 2019. The final version of the toolbox contained an information booklet and referrals to the NoMoreC website where supporting information, testimonials and instructional videos were available. The toolbox also contained condoms, fisting gloves, disinfectants, equipment for safe drug use and a booklet with practical information on HCV risk reduction (Figures 1 & 2). Table 1 shows how the IMB model constructs have been integrated in the intervention.

Figure 1. NoMoreC toolbox
The box contains a spray bottle (to clean surfaces where sexual activity takes place), sterilium® med hand disinfectant, hydrogen peroxide cleaning wipes (to disinfect surfaces where sexual activity takes place), gloves (for safe fisting), condoms (for safe sex), disinfection instruction card for hands and forearms, disinfection instruction card for dildos, toys & anal douche, a safe drug use box and booklet with information about HCV risk reduction. Republished from www.NoMoreC.nl under a CC BY license, with permission from the NoMoreC project group, original copyright 2018.

From March 2018, the toolbox could be ordered online free of charge from the NoMoreC project website (www.nomorec.nl/en/get-yourself-a-toolbox/). They were sent to addresses in The Netherlands only. Toolboxes were also available for pick-up at 17 locations in Amsterdam: at four fetish shops, a condom shop, the city central STI clinic of the Public Health Service, six HIV outpatient treatment centers, four GP practices, and a non-governmental organization (NGO) dedicated to improving the health and quality of life of people who use drugs (PWUD). Healthcare professionals were given the toolboxes to both distribute to MSM at risk and to discuss HCV prevention strategies with their clients.

Figure 2. Safe drug use box
A small cardboard box containing: snorting straws, a sharps container, syringes, needles, stericup® mixing cups and alcohol wipes. A sticker with the text "Never share drug-use equipment. Pick your own color" is stuck on the box. Republished from www.NoMoreC.nl under a CC BY license, with permission from the NoMoreC project group, original copyright 2018.

Table 1. Toolbox intervention components focusing on information, motivation and behavioral skills constructs and their aims.

Toolbox component	IMB-construct	Aim
Information booklet	Information Motivation Behavioural skills	Increase knowledge about: hepatitis C and its transmission, personal HCV risk, practical HCV prevention steps and their efficacy (response efficacy).
Referral to **instruction videos** on the NoMoreC website	Behavioural skills	Teach HCV prevention skills; Enhance self-efficacy to engage in HCV risk reduction behaviours
Referral to personal video **testimonials** about what men at risk do to reduce their HCV risk	Motivation	Develop positive attitude toward HCV risk reduction, improve motivation to engage in HCV prevention behaviours
Sterilium® med disinfectant for disinfection of hands and forearms	Motivation	Lower threshold to use the product
Disinfectant cleaning wipes for disinfection of surfaces where sexual activity takes place	Motivation	Lower threshold to use the product
Spray bottle for cleaning surfaces where sexual activity takes place before disinfection	Motivation	Lower threshold to use the product
Black latex gloves for safe fisting	Motivation	Lower threshold to use the product
Black nitrile gloves for safe fisting	Motivation	Lower threshold to use the product
Black condoms for safe anal sex	Motivation	Lower threshold to use the product
Illustrate instruction card: How to disinfect dildos, toys and anal douche	Behavioural skills	Teach disinfection skills; Enhance self-efficacy to engage in this HCV risk reduction step
Illustrated instruction card: How to disinfect ands and forearms	Behavioural skills	Teach disinfection skills; Enhance self-efficacy to engage in this HCV risk reduction step
Clip with suction cup to hang the instruction card for disinfecting hands and forearms on bathroom mirror	Motivation Behavioural skills	Lower threshold to use the product
Safe drug use box (Fig 2) for safe chemsex	Motivation	Lower threshold to use the product

Study design, participants and procedures

A mixed-methods study was conducted among toolbox recipients, using quantitative and qualitative data collection methods. Study participants of both the quantitative and the qualitative studies were included if they were at least 18 years of age, were a man who had sex with men, had received the toolbox and spoke the Dutch or English language. Firstly, online questionnaires were sent to toolbox recipients by email, 12 weeks after receipt of the toolbox in the period between March 2018 and March 2020. The questionnaire contained questions about HCV infection concerns, reasons for obtaining the toolbox, its use, context of use and impact of the toolbox on HCV risk reduction awareness (S1 Appendix). Answer options included yes/no, (3 and 5 point) Likert scales and lists with pre-defined answers (see S1 Appendix for complete questionnaire and answer options). Two members of the community group pre-tested the questionnaire and were asked to give feedback about the clarity of the questions and answer options. After the pre-test, some small revisions to the questionnaire were made. The validity of the questionnaire was not pre-tested. The questionnaire provided data that were subsequently used as the basis for the qualitative study, to further explore the themes of the questionnaires, build our understanding of the experiences of toolbox recipients and give more meaning to the quantitative findings.

The qualitative part of the study consisted of in-depth interviews with men who had received the toolbox from March 2018 onwards. Men who had ordered the toolbox online were given the option to leave their email address if they were willing to share their opinion about the toolbox in the future. Men who had received the toolbox in person from a healthcare professional or had picked it up at a fetish shop were asked if they could be contacted in the future, to share their experience with the toolbox. Those who agreed to be contacted left their email. In February 2020, all toolbox recipients who agreed to be contacted were sent an email outlining the research aims and request for participation in the study. 20 men reacted to the email and agreed to participate in the study. In the period March till May 2020, 15 out of 20 participants who had given their consent took part in an in-depth telephone interview, after which thematic saturation was reached. The interviews lasted 30 to 60 minutes. A topic guide (S2 Appendix) with open ended questions was used, to give participants the opportunity to describe their personal experiences with the toolbox. During the interviews, topics discussed included: experiences with hepatitis C, reasons for obtaining the toolbox, the actual use of the different toolbox products, motivations for, and barriers to the use of the toolbox products, context of use and experiences with the use of the products. We asked participants to reflect on the possible impact of the toolbox on their HCV awareness and on the participants' sexual and drug-use behaviours. We were interested to learn if participants had made any behavioural changes in their sex lives to reduce the HCV infection risk, based on the information in the toolbox, and what these changes

were. For example, did participants start cleaning and disinfect dildos and the area where sex takes place with the suggested products? Did participants who use drugs, safely administer their drugs by single use drug equipment and not share their equipment? Did men who enjoy fisting start using gloves and clean and disinfect their hands and forearms immediately after fisting? Furthermore, we asked the participants if the information provided by the toolbox assisted in making a personal HCV risk assessment. To thank the participants for their contributions they received a gift voucher of €20.

In addition, professionals from the STI clinic, HIV centres in Amsterdam and NGO for PWUD were contacted by email in February 2020, requesting their participation in our study. In March and April 2020, 7 out of 8 professionals who consented to be contacted took part in an in-depth telephone interview, after which thematic saturation was reached. The interviews lasted 10 to 30 minutes. Again a topic guide with open ended questions was used, to give professionals the opportunity to describe their experiences with the toolbox in their work setting. During the interviews, topics discussed included: their professional experience with hepatitis C, use of the toolbox in their daily practice and triggers and barriers to using the toolbox. Interviews were audio-recorded, transcribed verbatim and anonymized.

Data analysis

Simple descriptive statistics were used to analyze the quantitative data.

Interpretation of the qualitative data followed an inductive thematic analysis approach [19]. Two researchers (TP/JI) coded the transcripts independently and organized the data into open codes reflecting the major categories of information related to the study. Subsequently, codes were categorized into themes. Following the independent assessments of the transcripts, the researchers met frequently to compare and refine existing codes until they reached consensus on main themes and codes. MaxQDA Plus 2020 software (Release 20.0.8) was used for the qualitative data analysis.

Ethics

Participants gave their informed consent by clicking the consent box prior to completing the online questionnaire. Participants, who were interviewed gave their informed consent verbally, which was recorded on audiotape and documented by transcription. We received exemption from the Amsterdam University Medical Centre's medical ethical committee for extended protocol review (Reference number: W20_110 # 20.145).

Results

Participants

Toolbox recipients

A total of 174 men were sent an email with a link to an online questionnaire between March 2018 and March 2020. The questionnaire was started by 65 participants and completed by 49, resulting in a response rate of 37% (65/174) and a completion rate of 75% (49/65). Median age of participants was 47 years (IQR 40–57), 100% were MSM living in the Netherlands (Table 2). The majority was born in the Netherlands (88%). More than a third reported they were HIV negative (n = 18, 37%), of whom 78% were using PrEP. Twelve participants (24%) reported to have had an HCV infection in the past, of whom one participant reported more than one infection. The majority (83%) of men who reported a past HCV infection were HIV positive. Fourteen participants (29%) were worried about getting HCV infected, 22 (45%) were somewhat worried and 13 (27%) were not worried.

Table 2. Characteristics and concerns of toolbox recipients who filled out the online questionnaire (March 2018 and March 2020) or took part in an in-depth telephone interview (March-May 2020).

	Online questionnaires (n=49)	**Interviews (n=15)**
Socio demographic characteristics		
Age, years	47 (40-57)	48 (41-59)
Born in the Netherlands	43 (88%)	14 (93%)
Educated to college degree or higher[a]	NA	13 (87%)
Employed[a]	NA	12 (80%)
HIV and HCV status (self-reported)		
HIV status: positive/negative/ not disclosed	29 (59%) / 18(37%) / 2 (4%)	8 (53%) / 7 (47%) / 0 (0%)
Current PrEP use	14 (78% of HIV-negative MSM)	6 (86% of HIV-negative MSM)
Past HCV infection: Primary/ Re-infection	11 (22%)/ 1 (2%)	5 (33%)/ 0
Combined: Past HCV (re)infection and HIV positive Past HCV infection and HIV negative	10 (20%) 2 (4%)	4 (27%) 1 (7%)
HCV-related concerns		
I worry about getting hepatitis C	14 (29 %)	4 (27%)
I worry somewhat about getting hepatitis C	22 (45%)	7 (47%)
I do not worry about getting hepatitis C	13 (27%)	4 (27%)

Data are in n (%) or median (IQR). PrEP= Pre-exposure prophylaxis. NA=not applicable. a:Only asked during the in-depth interviews.

All 15 participants who took part in an in-depth telephone interview were MSM; 14 were living in the Netherlands and one abroad. All but one were born in the Netherlands. The median age was 48 years (IQR 41–59; Table 2). Most participants had at least a college education (87%) and were employed (80%). Almost half (47%) were HIV negative. Of the HIV-negative MSM, 86% were using PrEP. Five participants (33%), of whom 4 were HIV-positive, reported an HCV infection in the past. Four participants (27%) were worried about getting HCV infected, 7 (47%) were somewhat worried and 4 (29%) were not worried.

Healthcare professionals

Seven professionals took part in an in-depth interview. Two professionals worked at the STI clinic and four professionals at an HIV treatment centre in Amsterdam: five as registered nurses and one as nurse practitioner. One professional worked for an NGO dedicated to improving the health and quality of life of PWUD. The median age was 56 (IQR 44–58). All participants regularly saw MSM at risk of HCV in their care settings. They offered the toolbox to their clients and referred them to the NoMoreC project website. Professionals reported to have used the toolbox to discuss HCV risk and reduction during the consultations.

We present the combined results of the quantitative and qualitative analyses under four main themes: 1) Reasons for obtaining toolbox; 2) Toolbox usability and acceptability; 3) Context of use of the toolbox; and 4) Self-reported impact of the toolbox on HCV awareness and behaviour change. For each theme we commence with the questionnaire results and subsequently offer more in-depth related perspectives from the qualitative data. Perspectives of healthcare professionals are integrated into theme 1 (Reasons for obtaining toolbox).

Theme 1: Reasons for obtaining toolbox

Participants

The following reasons for getting a toolbox were given by 49 respondents of the online questionnaire: 35 (71%) wanted to reduce the risk of getting infected with HCV, 20 (41%) wanted to reduce the risk of transmitting HCV to someone else, 28 (57%) were curious to know the contents of the toolbox, 2 (4%) wanted to give the toolbox to someone else, 27 (55%) wanted to use it during sex parties and 6 (12%) got the toolbox because it was recommended to them.

During the in-depth interviews interesting paths and reasons for obtaining the toolbox were revealed. Exposure to information about HCV infection risk and the toolbox was sufficient to persuade men to try and use the toolbox:

"I was looking into PrEP. I wanted to start using that and my GP advised me to investigate what that involved. So then I came across the toolbox. That was the moment that I realised: yeah, I am at serious risk (of HCV). I didn't even know it existed. I only knew of hepatitis A and B and I got vaccinated for that."

"So when you were informing yourself about PrEP and getting PrEP care, you learned about the NoMoreC toolbox. Is that correct?"

– Interviewer

"Yes, that's correct."

– Participant 3

Some participants mentioned they wanted to inform their friends and sexual partners and openly talk about their sex lives in relation to HCV risk. They felt the toolbox could assist them in discussing these topics.

"You can reduce risk by openly discussing your sex life with friends. Or when you have a sex date or go to a place where you can have sex and just talk to like-minded people about it (HCV risk reduction). That box helps me a lot with that.... For example, I have shown the toolbox to a friend I regularly have sex with. I told him what the doctor had told me about all the products in the box. We then had a nice discussion about it."

– Participant 6

Healthcare professionals

Healthcare professionals indicated that the toolbox supports them in discussing HCV risk and HCV risk reduction. They want to inform their clients about HCV infection and how to prevent it. One professional explained:

"I always ask first: "We pay attention to hepatitis C, shall I tell you more about that? Would you like me to show you something?" And then I show the box. I tell them that they can have the box. But I always first show what it contains and say: 'Some things apply to you, other things do not apply to you, but this is all aimed at preventing hepatitis C or reducing the chance of getting hepatitis C."

– Professional 2

Some professionals mentioned that they showed the NoMoreC website first, including the risk reduction video's and subsequently explained the use of the

toolbox products. Many professionals used a client centred approach when discussing HCV risk, based on the client's knowledge about HCV and sexual behaviour. A lack of HCV knowledge, high-risk sexual behaviour and a past HCV-infection were motives for introducing the toolbox:

> *"During a consultation I find out what men know and what they don't know and how sexually active they are. Some say, 'No, a box like that is not for me' and others ask for more information. When I notice that someone is really interested, I tell them they can have the toolbox. For many men it (the toolbox) is an eye-opener and it also helps me discussing behaviour and risks. It helps because you can give very practical information."*
>
> *– Professional 4*

Some professionals indicated certain barriers for using the toolbox during consultations. The medical history form that is used during a consultation at the STI clinic is not inviting for introducing the toolbox and discussing settings in which it can be used as questions about group sex or visiting sex parties are not included. Several professionals also mentioned that sexual techniques such as fisting or sharing toys can be difficult to bring up during a consultation. Building a relationship with a client and seeing them more regularly, lowered this barrier and in some cases clients would bring up their sexual preferences more easily, as one nurse reported:

> *"Sometimes someone says: 'I like fisting, I go to fisting parties'. Then I will ask: 'have you heard of hepatitis C?' Also, I have information about hepatitis C on my table, so some men will ask about that and then it's an easy entry point to talk about it. But I don't just ask: 'Do you practice fisting?' or 'Do you share toys?'"*
>
> *– Professional 2*

Another professional mentioned that he did not have enough time to extensively discuss HCV risk reduction and explaining all the items in the toolbox. An HIV nurse explained that her focus is on HIV treatment and less so on HCV prevention:

> *"Well, I think it is a very handy box. It is just that our consultations are mainly focused on HIV treatment and if we notice that there is risk behaviour, we will of course discuss it. It just doesn't happen very often that we go into that matter (hepatitis C risk reduction) in more depth. So, it is not in my system to introduce that box."*
>
> *– Professional 7*

The use of the toolbox in the context of sexualised drug use was a problem for the professional who worked for an NGO dedicated to improving the health and quality of life of PWUD because he works with men who have recently stopped or want to stop engaging in chemsex. He was concerned that the safe drug use equipment in the toolbox would serve as a trigger for his clients who were battling addiction. Therefore, he did not hand out the toolbox to this group.

Theme 2: Toolbox usability and acceptability

Of 49 online respondents, 38 (78%) respondents used one or more toolbox products in the past 12 weeks and 41 (84%) intended to use products in the future. The use of the individual toolbox products since receipt of the toolbox is presented in Table 3.

Table 3 Use and intended future use of the individual products of the HCV prevention toolbox among 49 online respondents.

Toolbox product	**Product use** Number of participants who used the product, n (%)	**Future product use** Number of participants who intend to use the product in the future, n (%)
Disinfectant wipes	28 (57%)	41 (84%)
Sterillium® med hand disinfectant	25 (51%)	32 (65%)
Hand & lower arm disinfection instruction card	23 (47%)	17 (35%)
Condoms	17 (35%)	16 (33%)
Syringes for rectal administration of drugs	15 (31%)	19 (39%)
Sharps container	15 (31%)	17 (35%)
Latex gloves	14 (29%)	25 (51%)
Spray bottle	12 (24%)	27 (55%)
Nitril gloves	11 (22%)	15 (31%)
Syringes and needles for injecting drugs	11 (22%)	14 (29%)
Stericup® **mixing cups**	6 (12%)	11 (22%)

Number of participants who used (product use) and intend to use (future use) the product is given per product, and percentage of the total respondents (n=49).

The great majority of the participants (46/49, (94%)) rated the instructions of the toolbox as clear or very clear, two participants rated them as neither clear nor unclear and one participant reported to have not read the instructions. Responses to other questions related to toolbox usability and acceptability are shown in Table 4.

Table 4. Measures of toolbox usability and acceptability.

	Number of participants (%)
Usability	
The instructions for the items in the toolbox were:	
Very clear	21 (43%)
Clear	25 (51%)
Neither clear nor unclear	2 (4%)
I haven't read the instructions	1 (2%)
The instructions for disinfection were:	
Very clear	21 (43%)
Clear	24 (49%)
Neither clear nor unclear	1 (2%)
Unclear	1 (2%)
I haven't read the instructions	2 (4%)
Used the toolbox to start a conversation about hepatitis C with:	
Friends and sex partner(s)	18 (37%)
Friends (s) only	4 (8%)
Sex partner(s) only	5 (10%)
I have not used the toolbox to start a conversation	11 (22%)
No response	11 (22%)
Acceptability	
It is good that a box with a sharps container and drug equipment is included in the toolbox	
Strongly agree	25 (51%)
Agree	14 (29%)
Neither agree nor disagree	9 (18%)
Strongly disagree	1 (2%)
I would recommend the toolbox to a friend or sex partners	
Definitely	34 (69%)
Probably	11 (22%)
Maybe	3 (6%)
Probably not	1 (2%)

Responses of 49 participants who completed the online questionnaire 12 weeks after having received the toolbox.

During the in-depth interviews, the use of the products were typically mentioned in relation to the sexual technique and practices of the participant. All toolbox recipients had used or tried the toolbox products; some used many and others used few products, depending on their personal HCV risk. Participants continued to use the products and some had ordered several products again. It was highlighted

that some products were easier to use than others. Products that were received positively, were the disinfectant wipes, hand disinfectant and gloves. They were widely used and were highly accepted.

"The disinfectant wipes, I'm very happy with those. They are used everywhere. I now have them in my suitcase, always"

– Participant 6

"That (sterilium® med) disinfectant is really nice. It doesn't stink. I use it especially when I am done fisting. Then I wash my arms and use it."

– Participant 1

"Those black gloves have been a really good tip, I still use them when necessary."

– Participant 3

Mixed reactions were given about condoms and the safe drug use box. While some were happy the toolbox contained these products, others did not use them or disapproved of their inclusion.

"I used the condoms, I always have a box of condoms."

– Participant 3

"To be honest, I haven't used the condoms very often."

– Participant 13

"I have given away the condoms, because I don't use those. I use a different brand of condoms"

– Participant 8

"It (the safe drug use box) is of added value. The only thing I haven't used are the mixing cups. All the other things I have used."

– Participant 7

"I don't use (drugs) myself, but I know friends use. So it is nice to have."

– Participant 1

".. it contained needles and that sort of stuff. I was bit shocked by that. On the one hand, I think it is really good that it is included, but on the other hand I think: are you not promoting something?"

– Participant 13

Theme 3: Context of use of the toolbox

Of 49 online respondents, 28 (57%) used the toolbox during one-on-one sex, 22 (45%) used it during group sex with 3 to 4 men or at small sex-parties. Few respondents, 3 out of 49 (6%), reported the use of the toolbox at bigger sex parties with 5 men or more. One respondent used the toolbox in a group sex setting with both men and women and one respondent used it during tantric massages. A third of the participants (16/49 (33%)) discussed the use of the toolbox products with their sexual partner(s) before having sex, 10/49 (20%) discussed it with some of their sex partners, 12/49 (25%) did not discuss it and 11/49 (22%) participants did not answer the question.

During the in-depth interviews, all participants mentioned that they used the toolbox with sexual partner in a home-setting. The following participants described in what context they informed their sexual partners of the toolbox:

"I just use the toolbox during sex. This can be one-on-one or in a group. I always have the disinfectant to clean your hands after having played. And I tell people: 'Here is the dishwashing liquid and here is the disinfectant. This card shows how to clean your hands'."

– Participant 5

"When I organize a party, I just put that box in the room and say: 'Gentlemen, all kinds of tools for a safe party'."

– Participant 6

One participant described the use of the toolbox in the context of sexualized drug use:

"We slam (inject drugs) a lot. The fun thing is, the toolbox contains everything that you need to prevent needles and stuff being exchanged, because of those different colour syringes from the toolbox, everyone uses his own colour. So I put everything on the kitchen table with a note so everyone knows: this is my colour. Because of the toolbox we have started to work very systematically"

− Participant 7

Some participants expressed doubts in their ability to consistently apply the risk reduction strategies promoted by the toolbox, to their sex lives. It was felt that the products were easy to use in a home-setting but when having sex outside their home it was more difficult, as nobody would take the toolbox to a club or sauna. However, some reported that they compensate that by taking individual products when going out or on holidays.

"I don't take the toolbox to a club, that's a no brainer. But I pack some things in my suitcase when I go to a festival or when I travel, like gloves and hand disinfectant. But never the whole box, that is too much luggage."

− Participant 6

It was also reported that the use of the products was easier if the sex partner had seen the toolbox before and was already aware of the products.

Theme 4: Self-reported impact of the toolbox on HCV awareness and behaviour change

Of 49 online respondents, 23 (47%) strongly agreed, 23 (47%) agreed and 3 (6%) did neither agree nor disagree with the statement: "The NoMoreC toolbox gives me a better understanding of what I can do to reduce the risk of hepatitis C".

During the interviews, participants described that the toolbox had contributed to increasing their knowledge and awareness about hepatitis C and its transmission. They mentioned being able to better predict their risk of contracting hepatitis C and that the toolbox products helped them to reduce this risk. Both the toolbox products and being able to make different choices regarding the risks they took gave many participants a sense of safety as well as a feeling of being in control of their own health. The ability to protect others was also considered important. Participants reported that they had considered the preventive measures suggested in the toolbox, and implemented those that were attainable for them. Small changes in their behaviour were mentioned, such as the consistent use of disinfectant wipes or cleaning of sex toys.

"I used to clean things in a certain way, but dildos for example, you are supposed to put them in a bucket of water with bleach. I used to clean them with soap and then disinfect them. But with bleach it's better, because you get into the pores….so I know that now, and put the dildos in a bleach solution before I use them, so they are clean."

– Participant 5

One participant mentioned that since he had implemented the new hygienic measures and use of disinfectants that friends felt safe to join his sex parties. Others said that since exposure to the toolbox and being better informed about HCV risks, they had made a conscious decision to stop visiting sex parties. They felt by having one-on-one sex only, they could better control their risk:

"My sexual behaviour has changed. I don't go to big parties anymore. I prefer one-on-one, because I think that protects me more than the measures during sex."

"When you say: 'I have changed my behaviour', have you done that, based on the information in the NoMoreC toolbox?"

– Interviewer

"Yes, it has contributed of course. I have also visited the website a few times to inform myself. And you know, at the dates I have had in the past 2 years [after toolbox reception], I have always asked: 'How do you deal with hepatitis C?'."

– Participant 9

Next to the practical use of the toolbox products, the toolbox was also felt to provide good information to assess HCV risk and aid discussions about hepatitis C and risk reduction:

"That box helps a little to get a conversation going about hepatitis C, collect information and make a risk assessment. Especially in combination with the website."

"Can you explain how the toolbox helps you to get a conversation going?"

– Interviewer

"Well, someone will see the toolbox in my house and say: 'Hey, did you also get that box?' and then we will start talking about it. It sticks in people's minds when they have had that box, seen it or spoken about it. I hear from some of my friends: 'I have had

that box and I certainly learned something from it', we talk about the things (toolbox products) we use."

– Participant 2

Participants recommended or had given the toolbox to a friend and had used it to educate friends and sexual partners on risk reduction strategies. It was mentioned that there was still a lot of ignorance and lack of knowledge about hepatitis C among MSM.

> *"The good thing about that box is that I have talked about it with several friends, who didn't know anything about hepatitis C. And when I talked about hepatitis, they thought I was talking about A and B and said: 'I am vaccinated for that'. So, I thought it was a good thing that the toolbox got that conversation started."*
>
> *– Participant 13*

Discussion

Our study adds to the understanding of how MSM at risk of HCV and healthcare professionals respond to an innovative HCV risk reduction intervention such as the NoMoreC toolbox. The mixed-methods approach used, which combines quantitative and qualitative data, offers a valid and deeper understanding of participants' experiences with the toolbox and its possible impact on HCV awareness and behaviour change. We show that the toolbox gave men a sense of safety and control by being more aware of their HCV risks and by providing easier access to the right protection tools for themselves and others. Data collected from professionals, indicated that the toolbox is a useful aid to discuss HCV risk and risk reduction with clients.

The main motivation for MSM to obtain the toolbox is to reduce the risk of getting an HCV infection. All participants used the toolbox products to some degree and indicated good usability of the toolbox and found the instructions clear. Some participants indicated that the provision of the products has lowered the threshold for them to use these products, and facilitated their risk reduction behaviour. Furthermore, men reported the intention to use some of the products in the toolbox in the future. The use of the toolbox to openly discuss one's sex life, hepatitis C risks and related risk reduction strategies with friends and sexual partners was reported by many participants and suggests that the toolbox has contributed to encourage men to discuss sensitive HCV related topics. It has previously been shown that peers are more likely to influence behaviour, compared to mass media programs,

since they are able to build trust among fellow group members, which allows for open discussions on sensitive topics [20]. We believe that the 'viral' quality of the toolbox can contribute to further inter-peer dissemination of HCV knowledge and awareness. In the field of HIV prevention it has also been shown that peer interaction is an effective tool with long term effect for behaviour change among groups at high-risk of HIV [21].

The personal experiences with the toolbox demonstrate that the impact is different for each user. Some users reported that the NoMoreC toolbox has mainly impacted their knowledge and awareness about HCV, risk reduction and has helped them to assess their personal HCV risk. Others explained that they have changed their sexual behaviour based on the information provided. The use of the toolbox as a conversation starter was appreciated by many men, showing the wide acceptability of the toolbox within the target group. Furthermore, the users reported to use the toolbox products during one-on-one sex, group sex and chemsex, showing that the toolbox is suitable in a variety of contexts. Even though some dismissive reactions from users were reported in relation to the safe drug use box in the toolbox, almost one third of the quantitative study participants uses items for safe drug use and almost 40% intended to use them in the future. This suggests that the provision of safe drug equipment is important to some of the men in our target group and was reported in some cases to facilitate more structural safe practices of drug use. We were not surprised to find that some users were somewhat shocked by the safe drug use box, because its inclusion in the toolbox caused the most discussion during the development process [14]. We believe that the provision of safe drug equipment is important to some men of the target group, and even though controversial it does answer to a need.

Our results show that among professionals of the STI clinic and HIV treatment centres in Amsterdam the toolbox is a well-received intervention. They reported that the toolbox is a good educational tool that assisted them in discussions about risk reduction with clients. However, for some professionals, bringing up certain sexual techniques such as fisting and sharing toys can be difficult. In addition, consultation protocols that do not offer time or space to thoroughly discuss high-risk sexual behaviour and risk knowledge hamper the possibility to identify men at risk of HCV. This was also highlighted by a Swiss study among HIV-positive MSM with a past HCV infection, which showed that if professionals rely only on condomless anal sex with non-steady partners as a criterion to identify men at risk of HCV infection, disregarding information on other high-risk behaviours, such as fisting or group sex, they miss a proportion of MSM at risk [22]. We recommend the use of a comprehensive list of potential risk behaviours to identify men whose sexual and drug use behaviours increase their risk of HCV infection. The toolbox can assist

health professionals in identifying MSM at risk of HCV and prompt risk reduction conversations within consultation settings.

There are some limitations to this study. Participants of the quantitative study received the online questionnaire 12 weeks after the receipt of the toolbox, therefore we measured their experiences with the toolbox over a relatively short period. However, we did provide in our qualitative data the perspective of users who had received the toolbox up to two years prior to the interview, giving some insight into their experiences and possible behaviour change over a longer period of time. Future studies into the psychosocial, cultural, and personal factors that contribute to long term HCV risk reduction behaviour are recommended. In addition, support needs for MSM who are willing to change their behaviour and for MSM who are resistant to change are likely to be different. Therefore, studying both groups is essential for the development of future effective interventions.

We acknowledge that our study may have limited representativeness as data was collected only from participants who already ordered the toolbox and were willing to participate in the study, or from professionals who had reported use of the toolbox in the past. These participants may have been more positive about the intervention than those who did not want to obtain or use the toolbox, giving more favourable study results. Nevertheless, even among this highly motivated sample we uncovered some of the barriers to the (consistent) use of the toolbox.

Conclusion

This study has illustrated how a practical and relatively simple intervention, might contribute to facilitating HCV risk reduction. The HCV prevention toolbox was well-received by both MSM at risk and professionals. It contributed to raising awareness of HCV risk, implementing risk reduction strategies and it has given men a better understanding of the risk factors and how to reduce these. Our study showed how the toolbox was used to disseminate inter-peer HCV-related information and suggest the potential of the toolbox approach in the effort to impact the community norms. Furthermore, the toolbox can serve as an example intervention for countries that have similar sexual HCV transmission dynamics among MSM.

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Supporting information

S1 Appendix. Questionnaire sent to toolbox recipients

Introduction and consent

It's important that the Toolbox meets your needs. That is why we ask you to complete our feedback questionnaire. In it, we ask what you think of the Toolbox and the instructions that came with it, and your reason(s) for ordering the Toolbox. It will only take a few minutes, and we will be grateful for your help!

First, we ask for your permission to analyse your answers to the questions below. All data is treated with complete confidentiality and information is never linked to your name or (e-mail) address. You can read more about our research at www.NoMoreC.nl.

o I agree

Participant characteristics	**Question & answer options**
Age	How old are you? ..... years
Country of origin	Where were you born? Drop down list with countries
MSM	Are you a man who has sex with men? o Yes o No
HIV status	Are you HIV-positive or -negative o HIV-positive (I have HIV) o HIV-negative (I do not have HIV); I was tested less than 6 months ago o I don't know; my last HIV test was more than 6 months ago o I don't know; I have never been tested for HIV
PrEP use	Do you take PrEP (Pre-Exposure Prophylaxis)? o Yes o No
Past HCV infection	Have you ever been diagnosed with hepatitis C? o Yes, once o Yes, more than once o No, never
HCV infection concerns	**Question & answer options**
Concerns about HCV infection	Are you worried about getting hepatitis C? o Yes, I am worried o Yes, I am somewhat worried o No, I am not worried

Theme	Question & answer options
Motives for obtaining toolbox	Why did you decide to get a NoMoreC toolbox? o I want to reduce the risk of getting hepatitis C o I want to reduce the risk of transmitting hepatitis C to someone else o I was curious to know what was in the NoMoreC toolbox o To give to someone else o To use during sex parties o Someone recommended it to me o Other, namely...
Toolbox use	Have you used any of the items in the toolbox? o Yes o No
	Which items in the toolbox have you used or tried out? o Information booklet o Instructions for disinfecting hands and forearms o Sterillium® med o Blue Wonder® cleansing and disinfection wipes o Spray bottle o Latex gloves o Nitrile gloves o Condoms o Syringes for booty bumping o Syringes and needles for slamming o Stericup® mixing cups o Needle container
	Have you talked to your sex partner(s) before actually having sex about using any of these items, for example while chatting (online or face-to-face) or just before having sex? o Yes o No o Only sometimes

Toolbox use	Which of the items do you plan to use or continue using? o Information booklet o Instructions for disinfecting hands and forearms o Sterillium® med o Blue Wonder® cleansing and disinfection wipes o Spray bottle o Latex gloves o Nitrile gloves o Condoms o Syringes for booty bumping o Syringes and needles for slamming o Stericup® mixing cups o Needle container o I don't know yet o None of the above, because.....
Toolbox usability	Generally speaking, how clear were the instructions for the items in the NoMoreC Toolbox? o Very clear o Fairly clear o Neither clear nor unclear o Fairly unclear o Very unclear o I don't know; I haven't read the instructions
	How clear were the instructions for disinfection? o Very clear o Fairly clear o Neither clear nor unclear o Fairly unclear o Very unclear o I don't know; I haven't read the instructions for disinfection
	Have you used the NoMoreC Toolbox to start a conversation about hepatitis C with any of the following people? Friends o Yes o No Sex partner(s) o Yes o No

Toolbox acceptability	Do you agree or disagree with the following statement: 'I think it's good that a box with a needle container and drug equipment was included in the NoMoreC Toolbox.' o Strongly agree o Agree o Neither agree nor disagree o Disagree o Strongly disagree
	Would you recommend the NoMoreC Toolbox to a friend or fuck buddy? o Definitely o Probably o Maybe o Probably not o Definitely not
Context of use	In which setting have you used or tried out any of the items? o During one-on-one sex o During group sex/sex parties (3-4 men) o During group sex/sex parties (5 or more men) o Other, namely .....
Impact on awareness	Do you agree or disagree with the following statement: 'The NoMoreC Toolbox gives me a better understanding of what I can do to reduce the risk of hepatitis C transmission.' o Strongly agree o Agree o Neither agree nor disagree o Disagree o Strongly disagree

Toolbox usability	Generally speaking, how clear were the instructions for the items in the NoMoreC Toolbox? o Very clear o Fairly clear o Neither clear nor unclear o Fairly unclear o Very unclear o I don't know; I haven't read the instructions
	How clear were the instructions for disinfection? o Very clear o Fairly clear o Neither clear nor unclear o Fairly unclear o Very unclear o I don't know; I haven't read the instructions for disinfection
	Have you used the NoMoreC Toolbox to start a conversation about hepatitis C with any of the following people? Friends o Yes o No Sex partner(s) o Yes o No

The end. Pres 'send' to submit your answers. Thank you very much for your cooperation!

S2 Appendix. Interview guide

1. Research question and study population
2. General interview structure
3. Interview guides
3 a. Toolbox users
3 b. Healthcare professionals
4. Checklist

1. Research questions and study population

Research questions:

The aim of this study is to gather in-depth knowledge about the experiences with the toolbox.

1. What are the reasons for ordering a toolbox, intentions to use the products, the actual use, the user experience, motivations and/or barriers to using the toolbox products for MSM at risk of HCV?
2. What are the reasons, motivations and/or barriers to using and distributing the toolbox for healthcare professionals?
3. What are the reasons, motivations and/or barriers to distributing the toolbox for fetish shop owners?

In addition we aim to understand the experiences with the NoMoreC HCV testing service:

4. What are the reasons, intentions to use, the actual use and motivations and/or barriers to using the NoMoreC HCV testing service for MSM at risk of HCV?

Study population:

1. MSM who ordered, picked-up or received a NoMoreC toolbox.
2. Healthcare professionals from HIV treatment centers, STI clinic and GPs in Amsterdam who received NoMoreC toolboxes.

2. General interview structure

Introduction

- Personal introduction and introduction NoMoreC
- Purpose of interview/study:
- Finding out the reasons, motivation and barriers for using the NoMoreC Toolbox in order to improve the Toolbox and evaluate its use.
- There are no wrong answers, it is a conversation in which experiences are exchanged.
- It is not necessary to answer all questions. Given the theme of the research, personal and intimate topics may also be discussed during the interview. You

may also be asked about your personal opinion or experience. Not answering questions has no consequences for the continuation of the conversation.
- Results of the study will be used for scientific purposes (possible publication, data will be anonymized)
- Duration depends on what is being discussed but on average between 30 min and 1 hour.
- The interview will be recorded with a tape recorder, objection?
- In addition, notes are made, also anonymized, with code.
- Questions from the participant? Agree start tape?

Informed Consent (on tape)
- Content of the conversation (Use of and experiences with the NoMore C Toolbox)
- Consent to use and record the conversation
- Opt-out: it is possible to stop the interview at any time
- Study number + interview date

Structure depth interview
1. Introduction
2. Informed Consent
3. Demographic data/ background info/ history
4. Toolbox
5. Review toolbox
6. C-Test (for MSM only)

Closing conversation
Checklist: all topics answered, main themes addressed?
Extra addition from the participant?

Evaluation with participant
- Evaluation of the conversation from the participant's perspective
- Any questions from the participant?
- Incentive

Short summary of the conversation
Making a short summary of the conversation immediately after the interview, including a description of demographic data and the course of the conversation. Combine these with the transcription.

Transcription
Transcription by transcription agency.

3. Interview guides

3a. Toolbox users – max. 1 hour

Introduction
My name is ..... I work as a researcher at the GGD Amsterdam. I am currently conducting research into the experiences with the NoMoreC toolbox. The aim of the study is, based on in-depth interviews, to better understand what people think of the toolbox and how they use it and why. We want to improve the toolbox with the results of the research.

I would like to thank you very much for your willingness to participate in this study. Before we begin, I would like to go over a few things with you.

- The interview will be recorded. This is necessary to be able to properly understand your answers after the conversation and to process them afterwards. Your anonymity will be guaranteed and everything you say remains confidential. Your name or anything that could identify you will not be shared with third parties.
- The interview will take about an hour and aims to get a good picture of your experiences with the NoMoreC toolbox. If something is not clear to you or if you prefer not to answer; any question, feel free to say so.
- I will also take notes during our conversation to help myself remember some things.

If you agree, I would like to start recording now. Do you have any questions? The recordings can be stopped at any time, and you can ask questions at any time.

Informed consent
You have indicated that you are willing to participate in a study regarding the NoMoreC toolbox. In this conversation we discuss topics such as the risk of hepatitis C, prevention of an infection, the use of the toolbox and your opinion and experience with the toolbox. If you agree, we will record this interview. Data that is processed is always anonymized so that personal data cannot be returned to you. It is possible to stop the interview at any time. Do you agree?

Background information/History
Study number.....
I want to start with some general questions:
- Sex
- Age
- Origin
- Education
- Work: what kind of work do you do?
- Where do you live?

Questions about hepatitis C
- Could you tell us about your experience with Hepatitis C?
- Do you know how hepatitis C is transmitted?
- How do you estimate your chance of contracting hepatitis C?
- Are you concerned about this?
- Where does this feeling come from?
- Do you ever get tested for hepatitis C?

Timeline toolbox
Receipt toolbox
- Can you describe to me how you arrived at the Toolbox?
- What was your first reaction when you received the Toolbox?
- When was this?
- How did you experience this?

Use toolbox: barriers/facilitators
- Have you used the Toolbox?

Not used : What stopped you from using it?
Used: In which setting did you use the toolbox? Where and how?
- Did you use it after that?
- Can you think of any other ways you could use the Toolbox?
- Did you use it in a different way? Can you describe this?

- Did the Toolbox start a conversation about hepatitis C and its prevention?
- How has the Toolbox helped you to talk about hepatitis C?
- How did others react?
Have you recommended the Toolbox to others?

NoMoreC & C-test

- Besides the Toolbox, are you familiar with other parts of the NoMoreC project?
- Have you ever done the NoMoreC risk test? (this is the online questionnaire to determine whether you have been at risk)
- Have you ever ordered a C-test from NoMoreC? (to test for hepatitis C at home)

Did not order test:

- Have you ever considered ordering a test?
- What do you think about this possibility for testing?
- In what context would you possibly use it?
- Is there anything stopping you from doing a test via NoMoreC?

Did order test:

- What was the reason for ordering a test?
- Can you describe how the testing via NoMoreC went?
- How did you experience testing via NoMoreC?
- Have you recommended others to test via NoMoreC?

- What do you think of the NoMoreC project?
- How did you get to know the NoMoreC project?

Review toolbox products

Open the Toolbox and describe the products from the toolbox.

- Which items do you use and which items not?
- Are there any products you miss?
- How do you look at these products now?
- Are there any products you plan to continue using?
- In what context can you use it? In what context can you not use it? Moments or places?
- How would you like it if there was an HIV self-test in the box?

Ending the interview

- How did you experience the interview?
- Were there any ambiguities?
- Do you have any questions for me?
- Are there still things you would like to change?
- Do you have any points for improvement for me as an interviewer?

3b. Healthcare professionals – max. 30 minutes

Introduction
My name is ..... I work as a researcher at the GGD Amsterdam. I am currently conducting research into the experiences with the NoMoreC toolbox. The aim of the research is, based on in-depth interviews, to better understand what people think of the toolbox and how they use it and why. We want to improve the toolbox with the results of the research.

I would like to thank you very much for your willingness to participate in this research. Before we begin, I would like to go over a few things with you.

- The interview will be recorded. This is necessary to be able to properly understand your answers after the conversation and to process them afterwards. Your anonymity is hereby guaranteed and everything you say remains confidential. Your name or things that could identify you will not be shared with third parties.
- The interview lasts about half an hour and aims to get a good picture of your experiences with the NoMoreC toolbox. If something is not clear to you or if you prefer not to answer; any question, feel free to say so.
- I will also take notes during our conversation to help myself remember some things.

If you agree, I'd start shooting from now on. Do you have any questions? The recordings can be stopped at any time and you can ask questions at any time.

Informed consent
You have indicated that you are willing to participate in a study regarding the NoMoreC toolbox. In this conversation we discuss topics such as the risk of hepatitis C, prevention of an infection, the use of the toolbox and your opinion and experience with the toolbox. If you agree, we will record this interview. Data that is processed is always anonymized so that personal data cannot be returned to you. It is possible to stop the interview at any time. Do you agree?

Background information
- Sex
- Age
- Profession

Questions about hepatitis C
- Can you tell us about your experience with hepatitis C?
- How often do you see men who may be at risk for HCV?
- How is HCV discussed in your department/practice?

NoMoreC
- Have you followed the NoMoreC training?
- Have you done the E-learning? All 3 modules?
- What did you think of the training?
- Have you visited the NoMoreC website?

Toolbox
Use toolbox
- Can you describe how you use the toolbox?
- What are the triggers for you to use or distribute the Toolbox?
- When and how do you bring up the Toolbox?
- Does the Toolbox help you start a conversation about hepatitis C?
- Does the Toolbox help you to discuss risk reduction?
- How did men react when they received the toolbox?

Not used : What has stopped you from using or distributing the Toolbox?

Review Toolbox products
Open the Toolbox and describe the products.
- Are you missing something?
- What do you think about including an HIV self-test in the box?

4. Checklist

Introduction
- o Introduction researcher
- o Target research
- o Own experience: no wrong answers
- o Audio recording and anonymity
- o Taking notes

Informed consent
- o Number, recording, voluntary participation and possibility to stop, use of data
- o Demographics (m/f, age, position)

Interview

Toolbox users
- o Origin, education, place of residence

Chronology Toolbox
- o Receipt toolbox (how, when, experience)
- o Use toolbox (in which setting, continued use, way of use, reactions others)

NoMoreC & C test
- o Familiar with NoMoreC
- o C-test (use, reasons, experience)

Review toolbox
- o Go through toolbox

Professionals

NoMoreC
- o Training followed, e-learning, website visited

Toolbox
- o Use in practice, triggers for use, reactions
- o Barriers to use

Review toolbox
- o Go through toolbox

Chapter 7

General discussion

Effective interventions are urgently needed to reach the goal set by WHO to eliminate HCV as a public health threat by 2030 [1] and reach the global 2030 targets of 65% reduction in mortality, 80% reduction in incidence, ≥ 90% diagnosed and ≥ 80% treated, compared with the 2015 baseline [2]. In addition, 2021 WHO guidelines for countries seeking validation of elimination define the incidence target as an absolute annual incidence of ≤5 per 100,000 person-years in the general population and ≤2 per 100 person-years among people who inject drugs (PWID)[3]. To achieve global HCV elimination three key actions are needed: increased screening, strengthening access to treatment and prevention of primary infections and re-infections [1]. These actions can be challenging at a national level and require substantial effort and investment. For this reason, a more pragmatic approach has been suggested which breaks down national elimination goals into smaller goals for subgroups of the population, for which targeted treatment and prevention interventions can be implemented [4]. This concept is called micro-elimination. A modelling study suggests that HCV elimination among the population of MSM at risk likely requires early HCV diagnosis, immediate initiation of treatment and behavioral risk reduction strategies [5].

This PhD thesis explored the development, implementation and evaluation of the NoMoreC project, a multi-level intervention to reduce hepatitis C virus (HCV) transmission in men who have sex with men (MSM) in Amsterdam. It provides data on the co-creation process and on how the intervention was used and received by its users. In this chapter we reflect on the potential benefits of the intervention, how it can contribute to micro-elimination of HCV in MSM and the need to expand this intervention in the Netherlands and implement similar interventions in other countries. Furthermore, we will discuss recommendations for HCV testing and ways to assess HCV risk in a healthcare setting based on the results of our studies.

The subtitle of this thesis is “Innovative testing and prevention strategies in the Netherlands” because we believe that the developed testing and prevention strategies as well as the intervention as a whole are unique and innovative for a number of reasons. First, the intervention was co-created with members of the gay community, commercial stakeholders, stakeholders from within the gay community and health professionals, described in **chapter 4**. We believe that the active engagement of the community and stakeholders in the creation and implementation has been the main strength of the project. Second, the intervention used a multilevel approach, targeting different levels simultaneously. Various intervention components were developed to inform and have an impact on the at risk individual, the community, healthcare professionals, the context where high-risk behavior takes place, the individual diagnosed with HCV and sexual networks. Third, the intervention used a combination of web-based and face-to-face components that complemented each other. Fourth, the NoMoreC project was promoted by an

active team of volunteers, with a sex-positive tone, accepting lifestyle choices of MSM at risk of HCV, using online and face-to-face campaign strategies.

Targeted testing

Early detection and immediate (direct-acting antiviral) DAA treatment of recently acquired HCV infection is likely to reduce onward HCV transmission [6]. The WHO recommends general population testing in countries with a high HCV prevalence, without attempting to identify high-risk behaviors or characteristics [7]. In countries with low HCV prevalence among the general population, focused testing of specific key population at increased risk is advised. This involves testing of specific populations who are most affected by HCV, either because they are part of a high HCV prevalence population (e.g. some indigenous populations, migrants), or they have a high HCV risk because of risk behaviors (e.g. PWID, incarcerated people, MSM). In the Netherlands, with a low HCV prevalence of 0.1% a targeted testing approach will be the most impactful and cost-effective; focussing on migrants, prisoners, PWID, homeless people, and MSM. Cost-effectiveness studies of enhancing HCV screening of MSM with HIV, MSM using PrEP and MSM not using PrEP have been shown to be cost-effective [8]. A German study showed it was cost-saving to perform a one-time HCV screening among MSM with HIV [9]. Undertaking a one-time or yearly screening among all MSM in Belgium was cost-effective [10]. A Dutch study demonstrated that HCV screening and immediate DAA treatment of MSM with HIV is a cost saving approach that strongly reduces HCV transmission [11]. Scaling up testing and treatment services for all key populations is crucial to eliminate HCV. In this thesis the focus is on one key population: MSM. Therefore, in this chapter testing approaches in healthcare and community settings are discussed mainly for this group, but these may also be beneficial for other key populations. Ideally MSM with high-risk behaviors are screened regularly (e.g. quarterly) with a test that is sensitive and has a short window period. In addition, testing close after risk behavior will facilitate early diagnosis. Based on the results of our studies and the recent literature we will give recommendations for screening strategies that will contribute to elimination of HCV among MSM in the Netherlands.

Testing in healthcare settings

In the Netherlands, MSM living with HIV are screened for recently acquired HCV at the HIV treatment center by means of testing for alanine transaminase (ALT) twice a year [12]. MSM living with HIV who engage in behaviors associated with a high risk of HCV infection are additionally tested yearly using HCV serology [12]. If ALT levels are elevated or HCV antibodies are detected, HCV RNA testing should be

performed. MSM without HIV who use pre-exposure prophylaxis (PrEP) to prevent HIV are screened at the general practitioner (GP) practice, center for sexual health or can chose to take care of it themselves via online STI testing services. The current Dutch PrEP guidelines recommend screening for anti-HCV antibodies once a year [13]. If antibodies are detected, an infection is subsequently confirmed using an HCV RNA test. For individuals who have previously acquired HCV, the PrEP guidelines recommend yearly HCV RNA screening [13].

For marginalized populations at high risk of disengagement of care, such as PWID and homeless persons, a same-day treatment approach (i.e. starting DAA therapy on the same day of diagnosis) may increase the likelihood of patient engagement and retention in care. It has been demonstrated that PoC HCV testing has a greater effect on treatment initiation, when integrated into a simplified care model, where both testing and treatment are offered at the same location and, if feasible, on the same day, preferably in a community setting (discussed below)[14]. In the Netherlands, alternative HCV screening strategies such as one-step testing or the same-day test and treat approach may be particularly beneficial for marginalized populations to successfully engage them in care. Among the group of MSM living with HIV and MSM using PrEP disengagement from care is not expected as they are already in care and have regular appointments. However, earlier detection and initiation of treatment can be expected with a one-step testing strategy in this group as well.

As described in **chapter 3** of this thesis, a possible alternative to HCV RNA testing for the early diagnosis of recently acquired infection is HCV core antigen (HCVcAg). HCVcAg has been shown to be a reliable marker and correlates well with HCV RNA levels [15, 16]. We explore the application of the HCVcAg test for the diagnosis of recently acquired HCV infection during routine care of MSM at risk of HCV infection. We showed that the diagnostic performance of the HCVcAg test is reasonable, with a sensitivity of 90.6%. However, if the HCVcAg test would be used instead of HCV RNA, 9.4% of MSM with a recently acquired HCV infection would be missed. In the group of MSM with a reinfection the HCVcAg test had an even lower sensitivity of 80.0%. A Dutch modelling study by Popping et al. demonstrated that an HCVcAg monitoring strategy targeted only at a group of previously HCV-infected MSM living with HIV, while the rest of MSM living with HIV is monitored with ALT twice a year, is less costly compared with the current monitoring approach and will result in a reduction of HCV incidence and prevalence [17]. The model was based on a sensitivity of elevated ALT of 70-100% and HCVcAg of 90-100% [17]. These sensitivities were higher than we observed for the group of MSM with a previous HCV infection. Moreover, we found no statistically significant difference in sensitivity between HCVcAg and elevated ALT among the group of individuals with a reinfection and we would therefore not expect an impact on HCV incidence by replacing ALT by HCVcAg in this group. It would be interesting to repeat the modelling study of Popping et al. with the

sensitivities found in our study and expand the study to the whole MSM group at HCV risk including MSM with a previous HCV infection, and subsequently assess the impact of using HCVcAg on the HCV incidence and prevalence compared to the current monitoring approach. Furthermore, including updated cost prices of the different tests and the laboratory staff costs per test in the model will give a more precise cost estimate. Even though the HCVcAg may be less costly, the time required in the laboratory to perform the test and the availability of facilities to run HCVcAg tests may hamper the implementation of routine HCVcAg testing. As we found that using the HCVcAg test instead of HCV RNA would result in missing a proportion of MSM with a recently acquired infection, we do not recommend to replace the HCV RNA test with HCVcAg.

As risk factors associated with acute HCV infection are known and a risk assessment tool (the HCV-MOSAIC risk score [18]) has been developed to identify MSM at highest risk for an acute HCV infection, targeted testing is possible and recommended. Performance of the HCV-MOSAIC score was assessed in the development and in 3 validation studies, finding a sensitivity of the risk score of 70-100% among MSM with HIV [18]. A study by Jongen et al., evaluating the use of the HCV-MOSAIC risk score among MSM and transgender women living with HIV, showed a sensitivity of the HCV-MOSAIC risk score of 80.0% to diagnose HCV [19]. A recent study, assessing the HCV-MOSAIC risk score to predict HCV reinfection showed it to be useful for identifying MSM at risk of HCV reinfection as well [20]. The HCV-MOSAIC risk score is a practical tool that gives an indication of the extent of certain risk behavior and can assist healthcare professionals to identify MSM with high-risk behavior. Healthcare professionals at HIV treatment centers and centers for sexual health can discuss HCV risk behavior, aided by the HCV-MOSAIC risk score, with their clients to determine if HCV testing is needed. The incorporation of the 6 questions of the HCV-MOSAIC risk score (i.e. in the last 6 months, have you had condomless receptive anal sex; shared sex toys; fisted without a glove and in the last 12 months have you injected drugs; shared straws for snorting drugs; had an ulcerative STI? [18]) in the medical history form could be used for discussing and assessing individual risk. Because many healthcare professionals face time-constraints and some patients may not want to discuss their sexual behavior, an alternative could be to request patients to fill out the HCV-MOSAIC risk score prior to a consultation.

Community-based testing

Moving testing from the traditional healthcare environment to community-based and home-based settings can overcome obstacles to testing such as limited time, lack of transportation, stigma, lack of access to healthcare, and the need for multiple visits to a healthcare facility [21-23]. Community-based testing refers to an approach

which uses settings within the community, which are representative and used by key populations, to provided targeted testing services [24]. It includes outreach approaches in the general population and key populations, mobile testing, home-based testing, testing in parks, bars and other venues. It can be integrated in existing programs targeted at specific populations; for example providing HCV testing in homeless facilities or combining HCV testing with harm reduction programs for PWID.

Community-based testing approaches can improve access to HCV testing among MSM. It has previously been shown that client-initiated self-testing services for HIV are successful in increasing test uptake among MSM and trans people [25]. For the development of a low-threshold HCV testing service, we involved the target community through a co-creation process, which started with exploring their needs. In **chapter 4**, we presented these needs and recommendations from Amsterdam gay community members regarding HCV testing. The community group expressed their need for information about the different types of tests, where they could be tested, and more specifically for testing options for MSM without HIV other than at the GP practice. They also expressed their preference for a test that can also be used by men who have been infected in the past for diagnoses of reinfection (i.e. a test detecting the virus instead of antibodies), and to integrate HCV testing in a comprehensive STI screening package at the public health service of Amsterdam. Furthermore, the idea of a test that can be used at home was supported by the community and particularly convenient for MSM living outside of Amsterdam. Based on the community's recommendations, an anonymous HCV RNA home-based testing service was developed, using self-sampled dried blood spots (DBS). We believed a home-based testing service, outside clinical settings could decrease barriers to testing as it increases convenience, anonymity, perceived control over the testing procedure and their own health, and decreases time and efforts needed to visit healthcare facilities.

Prior to providing such a home-based test service, we assessed if people could adequately sample a DBS at home and if HCV RNA could be detected in these samples without compromising sensitivity of RNA detection. The evaluation of the self-sampling DBS technique for the detection of HCV RNA is described in **chapter 2**. Our study demonstrated that self-sampling dried blood spots at home is a feasible strategy for HCV RNA (and HIV-1 RNA) detection. We found self-sampled DBS can be used to measure HCV RNA with a high sensitivity (96.4%) and HCV RNA in DBS stored at room temperature is stable for a period of at least 21 days, making DBS a suitable collection device for home sampling in a real-life setting.

Our anonymous HCV RNA home-based self-sampling testing service, that was developed as part of the NoMoreC project was successful in reaching its target

population of MSM at risk of HCV. In **chapter 5**, we described the use and outcomes of the test service and reported user experiences. We demonstrated that the testing service effectively diagnosed HCV infections among MSM, with a very high HCV RNA positivity rate of 11%. The service scored high on measures of usability, acceptability and satisfaction among its users. The main reasons for men to use the service was that they wanted to be sure they were not HCV infected and because they were worried about an HCV infection. The relatively low price, time-saving and anonymous character were also seen as important. Interestingly, some users stated that they did not know another way of getting tested for HCV, which clearly showed that the NoMoreC test service made testing more accessible and increased uptake of testing. Furthermore, the use of the HCV-MOSAIC score prior to ordering the test gave men the opportunity to assess and reflect on their possible risk behaviors as described by one of the users of the test service:

> *"Before you order the test you have to fill in a questionnaire. Did completing the questionnaire give you a good assessment of your personal hepatitis C risk?"*
>
> *– Interviewer*
>
> *"Yes, it really did. Because when those questions are asked, you increase your knowledge about the topic. Well, let me speak for myself: when I have to answer those questions, then I get into the subject and find out what is risky behavior and what is not. So I find it informative to fill in such a questionnaire before testing."*
>
> *– User of NoMoreC test service*

Men who filled out the HCV-MOSAIC risk score on the NoMoreC website but did not proceed to ordering a test or decided to test elsewhere were not included in the test service evaluation. It would be insightful to understand their test behavior and barriers to use of the NoMoreC test service, and to evaluate whether they can be lowered to increase uptake and access to testing.

In addition to its use in a home setting, DBS-sampling is well-suited for mobile and outreach testing programs and has been accepted by WHO as an alternative approach to improve access to HCV testing and increase HCV testing rates [7]. As we showed our anonymous home test service was well accepted, expanding (anonymous) DBS testing to events in the gay community could further improve access to testing. In 2022, Young et al. showed the use of community-based HIV and HCV DBS testing, performed by peers, to be effective in Canada [26]. Gay, bisexual, trans, queer men and Two-Spirit and non-binary people were invited to test at Pride Festivals, and it was felt to be a low-barrier, cost-effective and simple way to quickly screen a large number of people. Such a community-based HCV testing approach,

could also be used in the Netherlands to improve access to HCV testing. Within the NoMoreC project, all prerequisites are present for a successful implementation in Amsterdam: active involvement and good relationships with the gay community and sex on premises venues (SOPVs) in Amsterdam. Furthermore, the volunteer team could combine their promotional activities with DBS sampling on-site at various events such as Queer & Pride Amsterdam, Leather Pride and Fetish Pride. Gay saunas and SOPVs can also be approached for either DBS sampling on-site, or as distribution points for DBS self-sampling kits. Discussions about such an approach had been held within the NoMoreC project team but implementation was hampered by a shortage of financial resources. Therefore, the first step would be to explore how to finance such an approach.

An alternative method to increase access to testing for MSM at risk is the integration of the HCV RNA testing service into an existing online STI testing service (e.g. Mantotman (31)). Being able to test for HCV, as part of a complete STI testing package, would improve testing convenience as suggested by users of the NoMoreC testing service.

The Covid-19 pandemic accelerated the demand for home-based testing for STIs as standard services were limited or unavailable. As a result, some centers for sexual health expanded their services with at-home specimen collection and self-testing for STI and HIV [27-30]. DBS sampling for HCV RNA screening for MSM at risk can easily be added to the repertoire of self-screening services. An HCV RNA home-based self-sampling test service is a valuable addition to existing sexual healthcare services, which may also reach men who are otherwise not tested. Therefore, we recommend the implementation of such a testing service by centers of sexual health throughout the Netherlands, and use targeted campaigns to encourage testing and raise awareness about available testing options. It has been demonstrated in a Chinese study by Ma et al. that the DBS method has goo d performance for simultaneously screening for antibodies to HCV, HIV and treponema pallidum [31]. By increasing the number of spots (e.g. 7 blood spots instead of 5 spots as in our service), HCV RNA measurement can be added to tests for other STIs.

DBS (self)-sampling for HCV diagnosis is also valuable in settings where there is a lack of access to laboratory facilities for HCV RNA measurements, or where timely delivery of specimens to a laboratory cannot be guaranteed. The use of DBS-based testing can improve the efficiency of testing in high endemic countries, resource-poor settings and for hard-to-serve populations. Ranjan et al. showed there was no deterioration of HCV RNA in DBS samples kept at high-temperature (≥37°C) for 15 days compared to those stored at 4°C [32]. Thus, DBS can also be used for collection and transportation of samples to test for HCV RNA in tropical countries.

Although DBS is a practical method for self-sampling, other capillary self-sampling options are available or currently being developed. Examples of capillary sampling options are the Mitra® device [33], the hemaPEN® [34], the Tasso+ device [35], the Microvette® APT[36] and the PBS-1000 blood collector [37]. It would be interesting to evaluate if these systems can effectively be used for self-sampling at home for HCV RNA testing, by assessing their ease of sample collection, sample volume, storage time, transportation possibilities, laboratory processing time and costs.

HCV prevention among MSM

Becoming aware of HCV risk and gaining knowledge about risk reduction strategies are the first steps in HCV prevention. Lambers et al. showed that during an earlier period of the HCV epidemic (2007-2009) the awareness of HCV was high among MSM participating in the Amsterdam Cohort Studies [38]. The study participants were knowledgeable about HCV transmission routes but had limited knowledge about complication of chronic HCV infection. A more recent Australian study also demonstrated high levels of HCV knowledge among MSM, but their knowledge about HCV treatment availability and their own HCV testing history was not as high [39]. A qualitative study exploring the awareness of, knowledge about and attitudes towards STIs among MSM in England and Wales, found that men perceived HIV and HCV the scariest infection, but more detailed knowledge was mostly lacking [40].

These studies suggest there is room for improvement for providing targeted information to MSM and educate them of their personal HCV risk, the consequences and strategies to prevent infection. As studies have shown that HCV transmission occurs mainly through sexual contact among MSM and what sexual techniques and settings pose the most risk for HCV infection [41, 42], tailoring information towards this population, raising awareness and giving specific advice was possible. With our intervention we aimed to increase knowledge and awareness at different levels, remove barriers and improve skills towards risk reduction behavior and promote regular testing. Primary prevention (preventing HCV infection) and secondary prevention (early detection of HCV infection) both contribute to reducing HCV incidence among MSM. **Chapter 4** and **chapter 6** described how the NoMoreC intervention targeted individual at risk, community, professional, context, individual with HCV, and network levels to influence the different determinants of our targeted behaviors. We demonstrated that the various web-based and face-to-face intervention components were well received and resonated with the gay community at risk of HCV infection.

The NoMoreC intervention approach was to give personalized advice based on risk behavior. We proposed 13 risk reduction strategies, supported by online

instructional videos and practical tools. During sexual activity we recommended the use of condoms during anal sex, the use of gloves during fisting, disinfection of hands and forearms after fisting, not to share dildos and sex toys, disinfect dildos and sex toys after use and not to share straws, syringes, needles, meth pipes or other drug-use equipment. Even though condom-use is crucial for the prevention of HCV, and other STIs, their consistent use poses a challenge to some MSM. The availability of PrEP for MSM resulted in a decrease in condom use during anal sex with casual partners by MSM on PrEP due to the decreased perceived HIV risk, as demonstrated in the Netherlands [43, 44] and other high-income countries [45, 46]. Furthermore, HIV transmission is effectively prevented by attaining an undetectable HIV RNA [47]. As the principle of U=U (undetectable equals untransmissible) is an effective strategy to prevent HIV [48], it has also reduced the need to use condoms for HIV prevention. A Dutch study among MSM showed that MSM living with HIV have a negative attitude towards condom use as a HIV risk reduction strategy [49]. Besides negative attitudes, another considerable barrier to risk reduction is the disinhibiting effect of drug use during sex. With the realization that some proposed risk reduction strategies were not attainable by some men and to accept lifestyle choices of MSM at HCV risk we chose for a harm reduction approach. The campaign slogan "CWhatYouCanDo!" was chosen, encouraging men to think about what risk reduction strategies they can and are willing to apply to their (sex) lives. Special care was taken to ensure the messages would neither evoke fear nor stigmatize the target population. The intensive cooperation with the community members allowed us to gather their perspective and was guiding in the choice of this slogan. We were successful in targeting MSM at risk and received positive feedback from users:

> *"These kind of prevention campaigns, usually pass me by. But this one (The NoMoreC campaign), well, you can probably hear it, this one really stuck."*
>
> *– User of NoMoreC toolbox*

Knowing that not all behavioral advice will be followed we still believe it is essential to give a complete picture of all possible risk reduction strategies, to make MSM aware and discuss them openly. In **chapter 6**, we described how one of the intervention components, the NoMoreC toolbox, facilitated risk reduction communication between peers and its potential to impact the community norms. The toolbox contributed to, raising awareness of HCV risk, a better understanding of the risk factors and how to reduce these and lower the threshold towards the application of risk reduction behaviors. Healthcare professionals reported the toolbox to be a useful aid for discussions about HCV risk and risk reduction strategies with their clients. They also stressed the importance of sharing all the risk reduction strategies with clients:

"I am 100% certain that you have to start from the theory and existing evidence about HCV risk. You may think that it is impossible for clients to do everything. For example all the cleaning (of toys, play area etc.), I wonder sometimes who is going to do all of that? Especially in the heat of the moment or on drugs. But it is good to share all the information and discuss it. I know from experience that clients are willing to make changes, they tell me: 'Well, I will do that, but I won't do that'. I have learned this working with the toolbox. Clients are willing to change their behavior: they may implement small measures that reduce the risk of hepatitis C. Not completely remove the risk, but reduce it."

– Healthcare professional

Nurses and nurse practitioners working at the center for sexual health and HIV treatment centers in Amsterdam felt supported by the NoMoreC toolbox to discuss HCV risk reduction with their clients. However, extensive discussions were not held with everyone as not all clients were perceived to be at HCV risk and because of time constraints (**chapter 6**). The incorporation of the HCV-MOSAIC risk score [18] in the medical history form during consultations can identify MSM at risk, as suggested by a nurse participating in our study. Subsequently, risk reduction can be discussed and a NoMoreC toolbox given or a follow up appointment can be made with a nurse specialized in sexual health. A more time-saving solution that we recommend is to ask clients to fill out the HCV-MOSAIC risk score prior to an appointment, using an online form or web application. This may also give the client some time to reflect on their sexual behavior and personal risk, which they can bring up for discussion during the consultation if they wanted to. Alternatively, a one-on-one discussion with a trained peer, a group discussion or group-based information session may be a good ways to share HCV information. In a group setting there is also room to exchange tips and tricks how to deal with HCV risk.

Our findings demonstrated that MSM are willing to reduce the risk of getting HCV. The impact of the NoMoreC toolbox differed between users: some users reported increased knowledge and awareness about HCV and indicated that the intervention aided them to assess their personal HCV risk, and some indicated easier risk reduction implementation (**chapter 6**). We did not evaluate which specific intervention component contributed the most towards risk reduction implementation and encourage more research to pinpoint this better. The support needs for MSM who are willing to change their behavior and for MSM who are resistant to change are likely to be different. By studying both groups and gaining better insight in their support needs, the intervention can be adjusted to better fit these needs and act upon factors that support risk reduction behavior. The impact of a behavioral intervention, implemented within the Swiss HCVree Trial, was also very different between users [50, 51]. In this trial, MSM living with HIV and on DAA

treatment were counseled to improve self-regulation of risks associated with sexual behaviors and sexualized drug use. Six to twelve months after the intervention some men indicated that they actively avoided their risks, some minimized risks and others accepted risks. This study showed that there is not a one-size-fits-all solution, and that interventions have to be tailored in approach and intensity to specific group needs and desires. Furthermore, the long-term effects of behavioral interventions need to be studied, including the effects of the NoMoreC intervention. We did not assess behavioral change, we only measured the use and self-assessed impact of the toolbox by MSM and healthcare professionals (**chapter 6**). It is imaginable that the impact of the intervention wanes over time, and that there is a need for repeated campaigns or the development of new interventions. On the other hand, it is also possible that once certain personally-acceptable behavioral strategies settle, that they endure over time. The NoMoreC toolbox is still being distributed today, and can also be ordered free of charge from the NoMoreC website. It would be interesting to assess the toolbox uptake over time and the geographic distribution in the Netherlands.

The Public Health Service in Amsterdam is currently conducting a multi-center randomized trial with 3 arms to evaluate the effect of a newly developed online tailored risk reduction behavioral intervention (based on the counselling intervention used in the HCVree trial [51], arm 1), a home based self-sampling HCV RNA testing intervention (based on the NoMoreC testing service, arm 2) and both interventions combined (arm 3) [52, 53]. The trial is conducted among men with a past HCV infection with risk of HCV reinfection in The Netherlands and France. Future results can inform the more structural implementation of one or both interventions if significant decreases in risk behavior are observed. The NoMoreC website can continue to complement new interventions as a good information source about risk reduction and to offer guidance. The website is kept active and is being managed by Soa Aids Nederland.

With the absence of an effective vaccine all efforts to prevent HCV infections continue to be focused on raising HCV awareness, reducing behavior associated with HCV infection, increasing testing, and early detection and treatment. However, a vaccine remains a very effective method to prevent infections and is essential to dramatically reduce HCV incidence [54]. The design of an effective HCV vaccine has been hampered by the huge genetic diversity of the virus and the molecular evolution of numerous virus escape mechanisms [55]. Recently, Sliepen et al. have unraveled the structure of the HCV E1E2 glycoprotein complex, which can provide a roadmap for the generation of a potent HCV vaccine [56]. Furthermore, new nucleic acid vaccine technologies, as used for COVID-19, may accelerate HCV vaccine research [57] and investment in vaccine research continues to be needed.

HCV micro-elimination among MSM

In the Netherlands, efforts are ongoing to achieve micro-elimination among MSM. Regular testing of MSM in clinical care followed by immediate DAA treatment in men with HCV have contributed to a reduction of acute infections [58] but micro-elimination has not been reached yet. HCV continues to be transmitted among MSM living with and without HIV [58-60]. This is in line with modelling studies showing that unrestricted access to DAA treatment without other prevention interventions leads to a reduction in infections, but is not enough to micro-eliminate HCV among MSM living with HIV [5, 61]. Targeted prevention approaches including behavioral interventions, such as the NoMoreC intervention, continue to be needed to reach micro-elimination among MSM. The NoMoreC intervention was successful in: targeting MSM at HCV risk, the development of a well-visited informative website, the implementation of an anonymous HCV test service that diagnosed HCV infections (positivity rate of 11%), giving trainings to health professionals and advice to SOPVs, and a risk reduction toolbox that was well received by MSM and healthcare professionals. Using a comprehensive approach that works towards increasing HCV knowledge and awareness, primary HCV prevention (through risk reduction strategies) and secondary prevention (through improved access to testing) are likely to contribute to reaching micro-elimination among MSM. The possible effect of the NoMoreC intervention on the epidemic has not yet been demonstrated, and direct impact will be difficult to assess. Dutch studies showing ongoing HCV transmission have used data up to and including 2018 [59, 62], the year the NoMoreC intervention was launched. A qualitative study exploring the awareness of, knowledge about, attitudes towards HCV and implementation of risk reduction strategies after exposure to the NoMoreC intervention components would be useful and could be related to HCV incidence data.

The International Collaboration on Hepatitis C Elimination in HIV Cohorts (InCHEHC) recently presented pooled data from Australia, France, the Netherlands, Switzerland and Spain over the period 2010-2019, showing a decline in HCV incidence among people living with HIV [63]. From 2015 to 2019 primary and reinfection incidence decreased by 49% and 27% respectively; reinfection incidence declined at a slower rate than primary incidence and its relative contribution to the current epidemic has grown since DAAs were introduced [63].

These downward trends in incidence are encouraging. Continued monitoring of risk behavior, HCV (re)infection incidence and HCV RNA prevalence among MSM and measuring progress in prevention interventions are crucial to assess if we are on track towards micro-elimination in MSM in the Netherlands. We recommend to continue to monitor the NoMoreC prevention intervention, by measuring its uptake (numbers of website visits, professionals trained, toolboxes distributed)

and evaluate its impact on behavior as mentioned before. Even with effective prevention interventions, like ours, next to routine testing and treatment of HCV (re) infections at a national level, HCV may be imported from other countries. A study by Koopsen et al. showed that the proportion of external introductions of HCV among MSM in Amsterdam has increased in recent years [64]. As HCV is not restricted by geographical borders and HCV continues to be transmitted through travel a broader European approach to HCV elimination among MSM is recommended. The NoMoreC intervention is a good example that can be transferred to other countries, provided the local gay community, health professionals and other stakeholders are involved in adjusting the intervention to meet the local needs and context. From the start of the implementation of the NoMoreC intervention we received various requests from European organizations to use components of the intervention. Interest was shown in the website, prevention toolbox, risk reduction cartoons, DBS self-sampling kit and self-sampling instructions. Some of the components were used as a source of inspiration and other components were translated and used in local prevention campaigns. The requests came from civil society organizations from Germany, Czech Republic, Spain, Italy and Sweden. The content of the NoMoreC website was translated in Spanish for the development of the ¡Disfruta Sin C! (Enjoy without C!) website, including the cartoons and personalized risk assessment tool [65]. The interest from other European countries shows there is a need and willingness to implement HCV prevention interventions. There is fertile ground for cooperation with other countries; sharing effective prevention interventions and online tools is advised. The connection of gay friendly cities offering gay night life and Pride festivals can be a good starting point, such as Antwerp, Paris, Berlin, Barcelona and London. Furthermore, the development of a European internet-based partner notification application can contribute to early testing of sexual partner(s) of a person with an HCV infection across borders.

We believe reaching micro-elimination in MSM is possible when there is close cooperation with the population at risk. Listening to their needs and tailoring interventions is key. The NoMoreC project has demonstrated that when healthcare professionals, public health professionals, microbiologists, researchers, professionals from civil society organizations, gay community members and other stakeholders join forces, tackling the HCV epidemic is possible. A wider implementation of our approach in the rest of the Netherlands is needed, combined with the necessary financial commitment. The NoMoreC co-creational approach may also serve as an example for other countries who want to develop culturally and context appropriate HCV interventions.

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Appendices

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Summary

Micro-elimination of hepatitis C virus among men who have sex with men
Innovative testing and prevention strategies in the Netherlands
This thesis focusses on the micro-elimination of hepatitis C virus (HCV) among men who have sex with men (MSM). The overall aim was to describe and evaluate innovative approaches to testing and prevention of HCV among MSM in Amsterdam, which may contribute to HCV micro-elimination in this population. In **chapter 1**, a general introduction on the topic of this thesis is given, including the natural history of HCV infection, advantages and disadvantages of different HCV diagnostic tests, HCV treatment, epidemiology and the micro-elimination concept. More detailed information is given about HCV among MSM, covering HCV transmission, risk factors and prevention. Furthermore, the concept of co-creation and the Information Motivation Behavioral skills (IMB) model are introduced. Co-creation and the IMB model were at the basis of the development of our multilevel intervention to reduce HCV transmission among MSM described in this thesis.

Chapter 2 focuses on the feasibility of self-sampling dried blood spots (DBS) at home. DBS self-sampling at home for the detection of HCV RNA was evaluated by comparing the detection of HCV RNA in plasma, lab DBS (DBS sampled in the laboratory) and self-sampled DBS. We showed that HCV RNA can reliably be detected in self-sampled DBS. In addition, we showed that HCV RNA in DBS stored at room temperature is stable for a period of at least 21 days. The use of self-sampled DBS can facilitate HCV diagnosis as it provides an opportunity for anonymous testing and improve access to HCV testing. It was concluded that self-sampling of DBS at home is a suitable technique for diagnosing acute HCV infections. In **chapter 3**, the application of another HCV screening tool was assessed. We studied whether HCV core antigen (HCVcAg) testing could be used to diagnose recently acquired HCV. It has previously been shown that HCVcAg instead of HCV RNA testing, can be used to diagnose chronic HCV. We found that the HCVcAg assay has reasonable diagnostic performance for diagnosing a recently acquired primary HCV infection, but the test sensitivity is not sufficient to allow its use as a screening tool for recent reinfection.

In **chapter 4** the development and implementation of a multilevel intervention to reduce HCV transmission among MSM in Amsterdam is described. We closely cooperated with health professionals and the gay community in Amsterdam. Commercial stakeholders and stakeholders from within the gay community were also involved at an early stage. The development process had four distinct phases: a needs assessment, stakeholder engagement, co-creation and implementation phase. The final intervention, NoMoreC, included web-based and face-to-face components as well as an anonymous HCV-testing service. It targeted six levels: the individual, community, professional, context, patient and network level. The

NoMoreC website provided information about hepatitis C, HCV transmission routes, risk reduction strategies, testing and treatment options and partner notification. The face-to-face component comprised a risk reduction toolbox, a training for health professionals and providing tailored advice to sex on premises venues. NoMoreC was promoted by an active voluntary campaign team. It was concluded that involving the community and stakeholders in the creation of the intervention was the main strength of the project. It resulted in an intervention with various components that resonated with the gay community at risk of HCV infection.

chapter 5 focusses on one of the NoMoreC intervention components, the anonymous HCV RNA home-based self-sampling test service. The service combined online HCV self-risk assessment with the possibility to test anonymously for HCV RNA. We demonstrated that the test service had a satisfactory uptake, a very high HCV RNA positivity rate of 11%, and was considered acceptable and easy to use by most MSM. **Chapter 6** describes the development and evaluation of another intervention component, the HCV prevention toolbox. The toolbox contained practical tools, to encourage HCV risk reduction in different settings. In addition, the toolbox was provided to professionals to facilitate discussions about HCV risk reduction with their clients. The HCV prevention toolbox was well-received by both MSM at risk and professionals. It contributed to raising awareness of HCV risk, implementing risk reduction strategies and it gave men a better understanding of the risk factors and how to reduce these. We demonstrated that the toolbox was used to disseminate inter-peer HCV-related information and suggested the toolbox approach has the potential to impact the community norms.

In the last chapter, **chapter 7**, the potential benefits and the transferability of the multilevel intervention are discussed. The outcomes of our studies are reflected upon and related to recent literature and developments in the HCV field. This chapter also contains recommendations on HCV testing in healthcare settings, community-based testing and HCV prevention among MSM, based on the results of our studies. It is stressed that targeted HCV prevention approaches including behavioural interventions continue to be needed to reach micro-elimination among MSM. We conclude that we believe reaching micro-elimination in MSM is possible when there is close cooperation with the population at risk. The NoMoreC project has demonstrated that when all stakeholders join forces, tackling the HCV epidemic is possible. A wider implementation of our approach in the rest of the Netherlands is needed, combined with the necessary financial commitment. The NoMoreC co-creational approach may also serve as an example for other countries who want to develop culturally and context appropriate HCV interventions.

Nederlandse samenvatting

Hepatitis C micro-eliminatie bij mannen die seks hebben met mannen
Innovatieve test- en preventiestrategieën in Nederland
Het onderwerp van dit proefschrift is de micro-eliminatie van het hepatitis C virus (HCV) bij mannen die seks hebben met mannen (MSM). HCV kan een infectie van de lever veroorzaken en wordt vooral via bloed-bloed contact overgedragen. Bij MSM kan HCV ook seksueel overgedragen worden. In dit proefschrift wordt een innovatieve benadering beschreven voor het testen en voorkomen van HCV bij MSM in Amsterdam. In **hoofdstuk 1** wordt een algemene inleiding gegeven over dit onderwerp. Dit hoofdstuk beschrijft het natuurlijke beloop van een HCV infectie, de voor- en nadelen van verschillende testen om HCV te diagnosticeren, de behandeling, de epidemiologie en het micro-eliminatie concept van HCV. Er wordt in detail ingegaan op HCV onder MSM, waarbij de transmissie, de risicofactoren en preventie van HCV in deze populatie worden besproken. Verder wordt het co-creatie concept en het Informatie-Motivatie-Gedragsvaardigheden (IMB) model toegelicht. Co-creatie en het IMB model lagen ten grondslag aan de ontwikkeling van onze multi-level interventie om de overdracht van HCV onder MSM te verminderen, zoals beschreven in dit proefschrift.

Hoofdstuk 2 richt zich op een bloedafname methode via een vingerprik (DBS), die men zelf thuis kan uitvoeren. Wij wilden onderzoeken of mensen zelf thuis een DBS kunnen afnemen en of hier vervolgens HCV RNA in gedetecteerd kon worden. Hiervoor werd HCV RNA in bloed, lab DBS (DBS bemonsterd in het laboratorium) en een zelf afgenomen DBS gemeten en vergeleken. We hebben aangetoond dat HCV RNA gedetecteerd kan worden in zelf afgenomen DBS. Daarnaast hebben we aangetoond dat bij kamertemperatuur HCV RNA stabiel blijft in DBS voor een periode van minimaal 21 dagen. Zelf afgenomen DBS kan de diagnose op HCV vergemakkelijken, aangezien het de mogelijkheid biedt om anoniem te testen en de toegang tot HCV-testen verbetert. De conclusie was daarom dat zelf thuis DBS afnemen een geschikte techniek is voor het diagnosticeren van acute HCV infecties. In **hoofdstuk 3**, hebben we onderzocht of de HCV core-antigeen (HCVcAg) test gebruikt kan worden om recent opgelopen HCV te diagnosticeren. Eerder onderzoek heeft aangetoond dat de HCVcAG test gebruikt kan worden in plaats van een HCV RNA test om chronische HCV te diagnosticeren. Wij lieten zien dat de prestaties van de HCVcAg-test voor het diagnosticeren van een recent opgelopen primaire HCV-infectie (eerste infectie) redelijk zijn. Echter, de gevoeligheid van de HCVcAg test is niet voldoende om hem als screeningstest voor een recente HCV herinfectie (een tweede of volgende infectie) te gebruiken.

In **hoofdstuk 4** wordt de ontwikkeling en implementatie beschreven van een multi-level interventie om de overdracht van HCV onder MSM in Amsterdam

te verminderen. We hebben nauw samengewerkt met zorgverleners en de Amsterdams homogemeenschap, en werden in een vroeg stadium commerciële stakeholders bij het project betrokken. Het ontwikkelingsproces kende vier fasen: een behoefteanalyse, het informeren en betrekken van stakeholders, co-creatie en de implementatiefase. De uiteindelijke interventie, NoMoreC, omvatte online en face-to-face componenten, alsmede een anonieme HCV-testservice. De interventie richtte zich op zes niveaus: het individuele, gemeenschaps-, professionele, context-, patiënt- en netwerkniveau. De NoMoreC-website gaf informatie over hepatitis C, transmissieroutes van HCV, strategieën om het HCV risico te reduceren, test- en behandelingsopties en partnerwaarschuwing. Onder de face-to-face componenten vielen een risicoreductie toolbox, het trainen van zorgverleners en het geven van advies op maat aan cruise bars en sekslocaties. NoMoreC werd gepromoot door actieve vrijwilligers, die online en offline campagnes ontwikkelden om MSM met risico op HCV te bereiken. Wij hebben geconcludeerd dat de nauwe samenwerking met de doelgroep en het betrekken van stakeholders bij de totstandkoming van de interventie essentieel was, en heeft geleid tot goed is ontvangen door de homogemeenschap en MSM met risico op HCV heeft bereikt.

Hoofdstuk 5 richt zich op één van de NoMoreC-interventiecomponenten, de anonieme HCV RNA thuis testservice. De testservice combineerde een online vragenlijst om persoonlijk HCV risico in te schatten met de mogelijkheid om anoniem te testen op HCV RNA. We hebben aangetoond dat de testservice redelijk goed gebruikt werd, een zeer hoge HCV RNA positiviteit van 11% en door de meeste MSM als gebruiksvriendelijk werd beschouwd. In **hoofdstuk 6** wordt de ontwikkeling en evaluatie van één van de andere interventiecomponenten, de HCV-preventie toolbox beschreven. De toolbox bevatte producten die gebruikt kunnen worden in verschillende settings om de kans op HCV overdracht te verminderen. Ook werd de toolbox verstrekt aan professionals om gesprekken met hun cliënten over HCV risico reductie te vergemakkelijken. De HCV-preventie toolbox werd goed ontvangen door zowel MSM als professsionals. Deze interventiecomponent heeft bijgedragen aan het vergroten van het HCV risicobewustzijn en de kennis over risicoreductie strategieën. Tevens gaf de toolbox mannen meer inzicht in hun persoonlijke risico's, en hoe deze te verminderen. We hebben aangetoond dat de toolbox werd gebruikt om HCV-gerelateerde informatie te delen tussen peers, en dat de toolbox-benadering mogelijkerwijs de gemeenschapsnormen kan beïnvloeden.

De belangrijkste bevindingen van bovenstaande studies worden in **hoofdstuk 7** besproken in het kader van recente literatuur en ontwikkelingen op het gebied van HCV. In dit hoofdstuk worden er aanbevelingen gegeven over het testen op, en voorkomen van HCV bij MSM op basis van onze resultaten. We benadrukken dat een gerichte HCV preventie aanpak, inclusief gedragsinterventies, nodig blijven om hepatitis C micro-eliminatie bij MSM te bereiken. Wij geloven dat micro-eliminatie

bij MSM mogelijk is wanneer er nauw samengewerkt wordt met de doelgroep en alle andere stakeholders. Wij pleiten voor een bredere implementatie van het NoMoreC project in de rest van Nederland, gecombineerd met de nodige financiële commitment. Onze co-creatie aanpak kan ook als voorbeeld dienen voor ander landen die HCV interventies willen ontwikkelen die goed aansluiten bij de lokale cultuur en context.

PhD portfolio

Courses	Year	ECTS
AMC Graduate School, Amsterdam, the Netherlands	2017	1.3
- Infectious Diseases	2018	1.9
- Qualitative Health Research	2019	2.1
- Advanced Topics in Biostatistics	2019	1.5
- Basic course on Regulations and Organization for clinical investigators (BROK)		
Public Health Service Amsterdam, the Netherlands	2017-2021	15.0
- Weekly PhD education (seminars, journal club, peer education, epidemiology class)	2017-2021	5.0
- Monthly Hepatitis Working Group	2017-2021	5.0
- Monthly HIV Journal Club	2020-2021	1.0
- Monthly COVID-19 Science update		
Virology Education, Utrecht, the Netherlands	2020	0.5
- COVID-19 Online educational program		
Seminars, workshops, masterclasses		
Public Health Service Amsterdam, the Netherlands	2018, 2020	0.2
- GGD Jaarseminar afdeling Onderzoek	2019	0.1
- GGD Onderzoeksdag		
Amsterdam UMC, the Netherlands	2018	0.1
- Virology Seminar	2019	0.5
- Castor Masterclass		
Dutch Association of HIV-Treating Physicians (NVHB), Amsterdam, the Netherlands	2018	0.1
- Wetenschappelijke vergadering		
The Netherlands School of Public Health and Care Research	2019	1.0
- CaRe days, Eindhoven, the Netherlands		
The European Aids Treatment Network (NEAT)	2019	0.5
- Acute HCV Consensus meeting, Amsterdam, the Netherlands		
Amsterdam Public Health research institute, the Netherlands	2019	0.5
- Pecha Kucha workshop		
Oral presentations		
- "Eliminating HCV from the HIV/HCV co-infected population: The Dutch experience" Hepatitis C elimination workshop 2017- Think global act local, Berlin, Germany. Co-presented with M. van der Valk and W. Zuilhof	2017	0.5
- "Eliminating HCV among men who have sex with men in Amsterdam" The Dutch Association of HIV-Treating Physicians (NVHB) meeting, Amsterdam, the Netherlands	2018	0.5
- "Innovative prevention for HIV and HCV" Dutch national conference STI*HIV*Sex, Amsterdam, the Netherlands Co-presented with M. Groot Bruinderink, M. van de Kerkhof, M. van der Valk and R. Verwijs	2019	0.5

- "The uptake of an innovative approach to reduce HCV transmission among MSM: the NoMoreC project" International Viral Hepatitis Elimination Meeting (IVHEM) 2019, Amsterdam, the Netherlands	2019	0.5
- "Evaluation of the NoMoreC test service and prevention toolbox" Dutch national conference STI*HIV*Sex, Amsterdam, The Netherlands	2020	0.5
- "Eliminating HCV among MSM in Amsterdam using an innovative approach" Gilead Science meeting, Breda, The Netherlands	2020	0.5
Poster presentations	**Year**	**ECTS**
- "Working towards eliminating HCV among men who have sex with men in Amsterdam using an innovative multilevel approach: the MC Free initiative" European Association of the Study of the Liver (EASL) monothematic conference 'Striving towards the elimination of HCV infection', Berlin, Germany	2018	0.5
- "Validation of home-sampled dried blood spots for HIV and HCV RNA load measurements" Global Hepatitis Summit 2018, Toronto, Canada	2018	0.5
- "Working towards eliminating HCV among men who have sex with men in Amsterdam using an innovative multilevel approach: the NoMoreC project" Global Hepatitis Summit 2018, Toronto, Canada	2018	0.5
- "Working towards eliminating HCV among men who have sex with men in Amsterdam using an innovative multilevel approach: the NoMoreC project" International Viral Hepatitis Elimination Meeting (IVHEM) 2018, Amsterdam, The Netherlands	2018	0.5
- "The uptake of an innovative approach to reduce HCV transmission among MSM: the NoMoreC project" Fast Track Cities 2019, London, United Kingdom. Presented by M. Prins	2019	0.5
- "Community engagement in NoMoreC, a project aiming to curb the HCV epidemic among men who have sex with men in Amsterdam" Fast Track Cities 2019, London, United Kingdom. Presented by P. Zantkuijl	2019	0.5
- "An innovative approach to reduce HCV transmission among MSM: the NoMoreC project" 12th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, The Netherlands	2019	0.5
(Inter)national conferences visited		
- The International Liver Congress, European Association for the Study of the Liver (EASL) Amsterdam, The Netherlands	2017	1.3
- Dutch national conference STI*HIV*Sex Amsterdam, The Netherlands	2017, 2018, 2019, 2020	1.0

- EASL monothematic conference 'Striving towards the elimination of HCV infection' Berlin, Germany	2018	0.6
- Global Hepatitis Summit 2018 Toronto, Canada	2018	1.3
- 22nd International AIDS conference Amsterdam, The Netherlands	2018	1.3
- Dutch National Hepatitis Conference Rotterdam/Utrecht, the Netherlands	2018, 2019	0.6
- International Viral Hepatitis Elimination Meeting (IVHEM) Amsterdam, the Netherlands	2018, 2019, 2020	1.8
- 3rd Annual Meeting of Amsterdam Public Health Amsterdam, the Netherlands	2019	0.3
- 12th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV) Amsterdam, the Netherlands	2019, 2020	0.6
Other academic activities		
Peer reviewer for BMC Infectious Diseases (*n*=1), Microbiology Open (*n*=1), AIDS and Behavior (*n*=1)	2020, 2021	0.6
	Total	**52.2**

Scholarships and awards	**Year**
- EASL Young Investigator award, Berlin, Germany	2018
- Scholarship to attend IVHEM, Amsterdam, the Netherlands	2018
- Scholarship to attend IVHEM, Amsterdam, the Netherlands	2019
- Scholarship to attend IVHEM, Amsterdam, the Netherlands	2020
Publications about my research	
- "Community involvement in de praktijk"(*Community involvement in practice),* for HEP15. Published on https://gilonline.nl/community-involvement-in-de-praktijk/ (accessed: 20 February 2023)	2018
- "Samen met msm groepen het risico op hepatitis c reduceren tot nul" (*Reducing the risk of hepatitis C to zero together with MSM groups),* for HEP15. Published on https://gilonline.nl/samen-met-msm-groepen-het-risico-op-hepatitis-c-reduceren-tot-nul/ (accessed: 20 February 2023)	2018
- "Gedurfde HCV-aanpak werpt vruchten af" (*Bold HCV approach pays off*), by N. van Esschoten, for MedNet Infectieziekten. Published on https://www.mednet.nl/nieuws/nomorec-in-amsterdam-gedurfde-hcv-aanpak-werpt-vruchten-af/ (accessed: 20 February 2023)	2019
- "Self-sampling for hepatitis C improves diagnosis rate in the Netherlands" by Keith Alcorn for Infohep. Published on http://www.infohep.org/Self-sampling-for-hepatitis-C-improves-diagnosis-rate-in-the-Netherlands/page/3550761/ (accessed: 20 February 2023)	2022

List of Publications

Prinsenberg T, Rebers S, Boyd A, Zuure F, Prins M, Van der Valk M, and Schinkel J. Dried blood spot self-sampling at home is a feasible technique for hepatitis C RNA detection. PloS One. 2020;15(4):e0231385.

Prinsenberg T, Zantkuijl P, Zuilhof W, Davidovich U, Schinkel J, Prins M, and Van der Valk M. Design and Implementation of a Multilevel Intervention to Reduce Hepatitis C Transmission Among Men Who Have Sex With Men in Amsterdam: Co-Creation and Usability Study. JMIR Form Res. 2020;4(9):e19100.

Prinsenberg T, Schinkel J, Zantkuijl P, Davidovich U, Prins M, and Van der Valk M. Internet-guided HCV-RNA testing: A promising tool to achieve hepatitis C micro-elimination among men who have sex with men. Journal of viral hepatitis. 2022;29(8):677-84

Prinsenberg T, Illidge J, Zantkuijl P, Bedert M, Prins M, van der Valk M, and Davidovich U. Usability, acceptability, and self-reported impact of an innovative hepatitis C risk reduction intervention for men who have sex with men: A mixed methods study. PloS One. 2022;17(2):e0263654.

List of contributing authors

M. Bedert — Department of Infectious Diseases, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

A. Boyd — Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, The Netherlands; HIV Monitoring Foundation, Amsterdam, The Netherlands

U. Davidovich — Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam, The Netherlands; Department of Social Psychology, University of Amsterdam, Amsterdam, The Netherlands

J. Illidge — Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, The Netherlands

M. Prins — Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam,, Amsterdam, The Netherlands; Department of Infectious Diseases, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

S. Rebers — Department of Medical Microbiology, Section of Clinical Virology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

J. Schinkel — Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology, Section of Clinical Virology, Amsterdam, The Netherlands

M. van der Valk — Department of Infectious Diseases, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; HIV Monitoring Foundation, Amsterdam, The Netherlands

P. Zantkuijl — Soa Aids Nederland, Amsterdam, The Netherlands

W. Zuilhof — Soa Aids Nederland, Amsterdam, The Netherlands

F. Zuure — Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, The Netherlands

Authors' contributions

Chapter 2
Dried blood spot self-sampling at home is a feasible technique for hepatitis C RNA detection

Name(s)	Contribution to the article
T. Prinsenberg	Substantial contributions to the conception and design of the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content and approval of the version to be published. Corresponding author.
J. Schinkel	Substantial contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.
S. Rebers	Acquisition of data, revising the article critically for important intellectual content and approval of the version to be published.
A. Boyd	Contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.
F. Zuure; M. Prins; M. van der Valk	Contributions to the conception and design of the work, revising the article critically for important intellectual content and approval of the version to be published.

Chapter 3
Application of the HCV core antigen test to diagnose recently acquired HCV infections among men who have sex with men in the Netherlands

Name(s)	Contribution to the article*
T. Prinsenberg	Substantial contributions to the conception and design of the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content.
J. Schinkel	Substantial contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content.
S. Rebers	Acquisition of data, revising the article critically for important intellectual content and approval of the version to be published.
A. Boyd	Contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content.
M. Prins; M. van der Valk	Contributions to the conception and design of the work, revising the article critically for important intellectual content.

* article has not yet been submitted for publication

Chapter 4
Design and Implementation of a Multilevel Intervention to Reduce Hepatitis C Transmission Among Men who have Sex with Men in Amsterdam: co-creation and usability study

Name(s)	Contribution to the article
T. Prinsenberg	Substantial contributions to the conception and design of the work, drafting the article and revising it critically for important intellectual content and approval of the version to be published. Corresponding author.
P. Zantkuijl; M. Prins; W. Zuilhof; J. Schinkel; M. van der Valk; U. Davidovich	Substantial contributions to the conception and design of the work, revising the article critically for important intellectual content and approval of the version to be published.

Chapter 5
Internet-guided HCV-RNA testing: A promising tool to achieve hepatitis C micro-elimination among men who have sex with men

Name(s)	Contribution to the article
T. Prinsenberg	Substantial contributions to the conception and design of the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content and approval of the version to be published. Corresponding author.
J. Schinkel; M. Prins; P. Zantkuijl; U. Davidovich; M. van der Valk	Substantial contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.

Chapter 6
Usability, acceptability, and self-reported impact of an innovative hepatitis C risk reduction intervention for men who have sex with men: A mixed methods study

Name(s)	Contribution to the article
T. Prinsenberg	Substantial contributions to the conception and design of the work, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content and approval of the version to be published. Corresponding author.
U. Davidovich	Substantial contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.
J. Illidge	Acquisition of data, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.
J. Schinkel	Substantial contributions to the conception and design of the work, analysis and interpretation of data, revising the article critically for important intellectual content and approval of the version to be published.
M. Prins; M. Bedert; M. van der Valk; P. Zantkuijl	Contributions to the conception and design of the work, revising the article critically for important intellectual content and approval of the version to be published.

Dankwoord

Birds flying high you know how I feel
Sun in the sky you know how I feel
Breeze driftin' on by you know how I feel

It's a new dawn
It's a new day
It's a new life
For me
And I'm feeling good

"Feeling good": geschreven door Anthony Newley & Leslie Bricusse, gezongen door Nina Simone

Yes, I'm feeling good! Ik heb bijna zes jaar gewerkt aan het project en onderzoek beschreven in dit proefschrift en wat voelt het goed om het nu af te hebben. Ik had het geluk om zowel te mogen werken aan het opzetten van een project als het doen van onderzoek. Dit proefschrift is het resultaat van het werk van velen. Het bedenken, uitrollen, en evalueren van de interventie was immers geen solo-exercitie en ik wil daarom stil staan bij eenieder die een bijdrage aan het project en/of onderzoek heeft geleverd. Ook wil ik iedereen bedanken die mij op een andere manier heeft gesteund om dit proefschrift af te ronden.

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About the author

Tamara Prinsenberg was born on February 15th 1973, in Rotterdam. She completed senior high school at Keira High School in Wollongong, Australia in 1990, after which she moved back to the Netherlands. She enrolled at Joke Smit College in Amsterdam to obtain VWO-certificates in 1991, and started her study Medical Biology at the University of Amsterdam in 1992. During her study she did research internships at the department of Tumor Biology of the Netherlands Cancer institute and at the department of Human Retrovirology of the AMC and graduated in 1997. Subsequently she studied Social and Cultural sciences for one year at the Free University of Amsterdam. In 1998, she started working at the headquarters of Doctors without Borders and studied part-time. She completed a post-graduate diploma in Community Health from the University of Applied Sciences Leiden in 2000. From 2000 to 2004 she worked in Maungdaw, Myanmar and Malange, Angola for Doctors without Borders and in Wow, Sudan for Action against Hunger. She moved to Melbourne, Australia in 2005, where she enrolled in a full-time Public Health course at La Trobe University. During this study she evaluated a surveillance system in Darfur, Sudan as part of her research thesis. In 2007, she completed her Master of Public Health, with a specialization in global health and infectious disease epidemiology. Upon her return in the Netherlands, she started working as a research coordinator, and later head of research of the Dutch Burns Foundation. During this time she also followed courses in epidemiology at the Free University of Amsterdam and in 2011 she obtained her registration as an Epidemiologist A. In 2014, she changed jobs to work at the Leprosy Research Initiative, where she worked on improving research capacity in leprosy endemic countries and mobilize financial resources for leprosy research. Tamara started her PhD trajectory at the Amsterdam UMC, location AMC and the Public Health Service of Amsterdam, in April 2017, under the supervision of prof. Marc van der Valk, prof. Maria Prins, dr. Janke Schinkel and dr. Udi Davidovich. The results of her work are presented in this thesis.