

Study Protocol and Statistical Analysis Plan

**Approved by a human subjects protection review
board: 20th February 2016**

Study Title:	The impact on linkage-to-care of an alternative hepatitis C screening method in PWID
Approved by a human subjects protection review board	02/20/2016
Institution Name	Hospital Universitario Infanta Leonor Centro Nacional de Microbiología – Instituto de salud Carlos III

2.1 Objectives & Hypotheses	<p><u>Hypothesis:</u> The development of alternative methods of screening for HCV infection in persons who inject drugs (PWID), as part of a harm reduction strategy at “Cañada Real” shantytown (Madrid), may facilitate diagnosis of the disease in this population, increase the frequency of referral to hospital and administration of appropriate HCV treatment and thus reduce the prevalence of HCV.</p> <p><u>Objectives:</u> The overall objective of this project is to evaluate whether the implementation of an alternative method of HCV screening in PWID from "Cañada Real " (Madrid) can improve the localization of HCV-infected patients for subsequent referral to the Hospital, administration of HCV antiviral therapy and cure of hepatitis C. The effectiveness of the intervention in each of the phases of the project will be assessed.</p> <p>Main objectives:</p> <ol style="list-style-type: none"> 1. To develop and validate an alternative method of screening for active hepatitis C virus (HCV) infection based on collection of capillary blood via finger pricking and Whatman™ cards (dried blood). 2. Implementation of a screening program in PWID from "Cañada Real" (Madrid) aimed at early HCV detection (diagnostic) and early HCV treatment as part of a harm reduction strategy, and 3. To assess the effectiveness of our alternative HCV screening test in PWID through evaluation of the impact on the patients' health (referrals to health centers, number of treated patients, and the percentage of cure). <p>Secondary objectives:</p> <ol style="list-style-type: none"> 4. Estimation of the prevalence of HVC active infection or HCV resolved spontaneously with the methodology developed. 5. To estimate the prevalence of other chronic viral infections (eg, HIV, HBV, and HDV) that are relatively common in PWID. 6. To estimate the prevalence of active HCV infection 24 months after the first intervention in “Cañada Real”. 7. To assess factors associated with HCV infection and transmission through an epidemiological survey, which will collect demographic data and risk behaviors.

**2.2
Background &
Rationale,
Significance of
Selected
Topic &
Preliminary
Data**

HCV infection is a major health problem worldwide. The main source of infection is currently parenteral drug use, especially in more developed countries. HCV infection is the main cause of liver-related death and the principal indication for liver transplant in industrialized countries. Increased knowledge of the molecular mechanisms involved in the cell cycle of HCV has made it possible to develop a new family of drugs known as direct-acting antivirals (DAAs). Treatment courses are now short and very safe. In addition, dosing is convenient and efficacy and cure rates are high.

Spain has one of the highest HCV infection prevalence values in Europe. The estimated prevalence of HCV antibodies is 1.7%, and the prevalence of positive viral load in adults is 1.2%, that is, 472,000 adults with active infection. However, it is estimated that only 40% of HCV-infected persons in Spain have actually been diagnosed, probably because conventional screening strategies have not been widely applied and have proven to be of limited efficiency both in the general population and in hard-to-access risk groups. Despite the extent of this health problem, the true prevalence of HCV infection in our area is not known. The recently established National Hepatitis C Plan opened a line of investigation aimed at quantifying the problem, describing the epidemiological characteristics of those infected, and establishing preventive measures. The National Hepatitis C Plan, which came into force on April 1, 2015, has been a definite boost in the treatment of hepatitis C in Spain. In the first year of the Plan, more than 40,000 patients with HCV have been treated, acquiring similar cure rates to those reported in clinical trials. HCV therapy in Spain is widely available and PWID are considered to be a priority population.

Screening for HCV infection is cost-effective, since early identification helps limit the transmission of the virus and enables patients to benefit from drug therapy and non-pharmacological measures that prevent them from developing advanced forms. In this context, early diagnosis of infection is becoming increasingly important, as is the use of DAAs, which represent the first step on the road to eradicating HCV infection. Screening for HCV infection is currently based on serological testing followed by further extraction to confirm active infection based on the HCV-RNA load in blood. Several international organizations have stressed the need to find new ways of reaching out to patients in the form of alternative tests for detecting active infection (presence of HCV-RNA in blood) in order to enhance screening for HCV infection, diagnosis, referrals to health centers, and treatment.

Intravenous injection continues to be the most frequent route of transmission of HCV in Europe (79% of new infections in 2012) and is the key driving force in the perpetuation of the epidemic. The percentage of undiagnosed PWID is probably greater than in the general population, given that this vulnerable group has little access to the health system. Accordingly, the World Health Organization (WHO) stresses that all HCV-infected persons should have equal access to prevention, diagnosis, and treatment and that special efforts must be made with regard to more inaccessible population groups.

Modeling studies indicate that the only way the prevalence of HCV infection can be substantially reduced is through the early publication of across-the-board public health strategies that include significant increases in the frequency of diagnosis and access to treatment³.

PWID are at high risk from blood-borne infections, including human immunodeficiency virus (HIV) and HCV through the sharing of needles and injection equipment. Harm reduction refers to policies, programs and practices that aim to reduce the harms associated with the use of drugs in people unable or unwilling to stop. Harm reduction began to be discussed frequently after the threat of HIV spreading among and from PWID was first recognized. However, similar approaches have long been used in many other contexts for a wide range of drugs. There is persuasive evidence from the literature that harm reduction approaches greatly reduce morbidity and mortality associated with drug use and combined prevention measures (Needle and syringe programmes & opioid substitution therapy) contribute to the reduction of the spread of

HIV and HCV infections. Although there is concern that HCV re-infection may negate the potential benefits of treatment, the reported rates of reinfection following successful HCV treatment among PWID are low, especially when combining high coverage of harm reduction measures.

Cañada Real is a major shantytown on the outskirts of the city of Madrid. It is estimated that 90% of the illegal drugs in the Madrid region are sold and consumed in Sector VI of Cañada Real and that between 4000 and 6000 people per day go to this shantytown for their drug doses. The Anti-Drug Agency (Agencia Antidroga) runs a daily mobile unit in the area as a harm reduction strategy. The remit of the unit is to minimize damage from drug consumption by taking health-related, psychological, and social measures. It deals mainly with outreach drug consumers (individuals who are outside the reach of the health system and have great difficulty accessing it). This harm reduction unit identifies drug users at risk, offering them needles, syringes, opioid substitution therapy and screening for HIV and HCV. When a PWID tests positive for HIV or HCV, he is referred to Infanta Leonor Hospital for appropriate therapy. The harm reduction team accompanies the drug user to hospital and ensures his follow-up and adherence to therapy. Recent hepatitis C test and care programs have identified the use of patient navigators or care coordinators to be an important intervention in overcoming challenges to linkage to and retention in care

In Spain, prevention and screening of HCV infection is ensured mainly through primary health care. Infection is diagnosed using serological tests followed by retests to confirm active infection. The tests are used to detect HCV RNA in blood and are carried out at specialist health centers. However, a large number of people at high risk of infection do not visit health centers regularly, and even when they do, the centers lose track of a very high percentage of patients in the follow-up stage. Thus, a simple screening method or an alternative diagnostic test that can be used in situ could make testing more widely accepted and improve rates of diagnosis, referral, and treatment.

There are several quick, highly specific, highly sensitive tests on the market for detecting HCV antibodies. The tests are fast, simple, and provide results on site. However, the need for further testing to confirm results usually involves a number of visits for testing and collection of results. Consequently, many patients are lost during follow-up. Screening strategies should therefore be capable of ensuring that active infection is correctly diagnosed. Changes in the screening algorithm have recently been proposed so that HCV-RNA can be detected directly, especially in relatively inaccessible high-risk populations such as PWID.

Here, we seek to validate and implement an alternative method to screen for HCV and other viruses in an attempt to facilitate diagnosis of HCV infection, increase referrals to specialist health centers, and thus increase treatment coverage in our area. The alternative screening method proposed requires five drops of capillary blood to be collected by finger pricking onto Whatman cards. This method is more widely accepted than venous extraction and has enabled diagnosis rates to be improved by two- to six-fold among PWID at community services in the United Kingdom.

Several studies have shown the validity of dried blood samples for detecting HCV RNA using amplification techniques. Moreover, dried blood samples can also be used to perform HCV genotyping and phylogenetic studies, which can reveal transmission networks. However, although using dried blood samples offers advantages in terms of diagnosing active infection, it does not preclude the need to call individuals back in order to give them the results and provide them with suitable advice, as samples are sent to the laboratory and the results may take some time to come through.

Screening via finger pricking using Whatman cards is simple and cheap and can be applied with only minimal training. It obviates the need for venipuncture and syringes, requires only minimal amounts of blood, and takes up little storage space. Consequently, samples are easy to carry and handle. Proactive screening and active approaches to users will enable us to quantify the problem, learn the epidemiological

	<p>characteristics of HCV-infected patients, and thus establish more suitable preventive measures and a circuit of referral to specialist centers for confirmation of diagnosis and treatment.</p> <p>Screening, diagnosis and treatment of HCV in PWID, should be part of a harm reduction strategy. Treatment of HCV infected PWID should be delivered in a multidisciplinary care setting with services to reduce the risk of reinfection and for management of the common social and psychiatric comorbidities in this population. More frequent diagnosis, new methods that prevent loss of tracking, and access to antiviral treatment are all strategies that must be implemented jointly if the prevalence of HCV infection in our setting is to be reduced.</p>
2.3 Study Design	<p>TYPE OF STUDY: Multi-center: "Infanta Leonor" Teaching Hospital (HUIL), National Microbiology Center–Carlos III Health Institute (CNM-ISCIII), and the Harm Reduction Unit (HRU) (Madrid Positivo and the Anti-Drug Agency).</p> <p>DURATION OF THE STUDY: 24 months.</p> <p>DESIGN OF THE STUDY: Cross-sectional.</p> <p>SCOPE OF THE STUDY: The study population comprises persons who visit the Cañada Real Galiana shantytown on the outskirts of Madrid, where 90% of illegal drugs in the region are sold and consumed. It is estimated that between 4000 and 6000 people per day visit the shantytown for their dose. The regional government's Anti-Drug Agency has a mobile HRU operating daily in the area. The unit provides screening tests to anyone who is willing to take them, regardless of whether they are already aware of their serological status or not. Screening includes education on prevention of HCV infection and other transmissible diseases.</p> <p>PHASES OF THE STUDY</p> <p>1) <u>PHASE I</u>: Setting and calibration of the laboratory techniques needed to carry out the study. The screening method will be validated with dried blood spots from Whatman 903™ cards taken from volunteer patients at HUIL. The results will be compared with the results of a venous blood sample taken from the same individual at HUIL. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests will be determined.</p> <p>2) <u>PHASE II</u>: Screening for HCV and other viruses (HIV, HBV, HDV) using dried blood samples on Whatman™ cards in subjects from Cañada Real based on the results of the laboratory tests performed in phase I. The data and samples will be collected by the HRU. All the samples collected will be sent by courier to the CNM-ISCIII for processing and analysis.</p> <p>3) <u>PHASE III</u>: Evaluation of the effectiveness of the intervention. Subjects who had a positive result in the screening performed in Cañada Real Galiana will be contacted and offered the possibility of referral to HUIL, where they will have access to standard confirmation tests. Here, test accuracy will be evaluated at population level. Patients will have access to HCV treatment and will be followed for assessment of the impact of the program on patients' health (referrals to health centers, percentage of treated patients, and the percentage of virological response). As part of the harm reduction program patients will be screened periodically after therapy to detect HCV reinfection.</p> <p>COLLECTION AND PROCESSING OF SAMPLES</p> <p>Biological samples will be collected according to the study protocol as dried blood spots on Whatman 903 cards (50-75 µl of blood). The blood samples obtained will be processed according to the protocol for handling samples set out in Annex 1 of the</p>

protocol.

The Whatman 903 cards used to collect whole blood will be handled by healthcare professionals. The device consists of a small card with five circles for collecting one drop of blood each. The card is attached to a record card where demographic information on the individual being tested is recorded. A small amount of capillary blood from the user's ring or middle finger is collected on the sample collection area and then allowed to dry at room temperature for three hours. The card is then made ready for transportation to the screening laboratory. It must be taken to the laboratory within 15 days of collection.

Samples will be sent to the CNM-ISCIH for laboratory testing. The cards will be stored at -80°C .

PHASE I: INTERNAL VALIDATION AND REFINING OF THE TECHNIQUE

Once the ethics committee has approved the study, samples will be obtained by finger pricking using Whatman 903TM cards at visits to the Internal Medicine department of HUIL. All patients visiting the department, for whom recent analytical data (serology results for HCV, HIV, HBV, and HDV, as well as HCV-RNA viral load and HCV genotyping collected during the previous 15 days) will be asked to take part in the internal validation study. Patients whose analyses were performed elsewhere or are not recent will not be included. Healthy individuals, i.e. those who have tested negative for HCV, HIV, HBV, and HDV will also be included. Individuals will be included in the validation consecutively until the preset number is reached (see below).

A total of 100 patients and 50 healthy individuals will be included to validate the screening test. Successive samples will be taken, and patients with genotypes 1a, 1b, 2, 3, and 4 will be included. National data indicate that the distribution of the most frequent genotypes in Spain is as follows: 1b, 44%; 1a, 26%; and 3, 20%. Therefore, these will be the genotypes most widely represented in our study. The 50 healthy individuals (negative for HIV, HBV, HCV, and HDV) will be obtained from the family members accompanying patients on medical visits, who will be invited to take part in the study. One dried blood sample will be taken by finger pricking and another simultaneous sample will be taken by venipuncture. The venipuncture sample will be processed through the usual channels at the center.

Participants will be given an information leaflet and a specific informed consent form. Samples will be processed according to the study protocol (Annex 1). Clinical and epidemiological data will be recorded in a database that can be accessed by researchers from the HUIL and the CNM-ISCIH. The variables shown in section 2.4 will be recorded. This file has been included in the Register of Data Files of the ISCIH.

ELISA: A disc with a sample of dried blood will be placed in a microtube containing 300 μl of 0.5% buffer solution (PBS/BSA) and processed at $4-8^{\circ}\text{C}$ for 18-24 hours. After incubation, elutes from the dried blood samples will be tested for the presence of antibodies against HCV, HIV, and HDV, as well as for HBV surface antigen (HBsAg). Negative samples will not undergo further testing and will be considered non-infected. Positive samples will be tested using real-time PCR to confirm the positive results.

Real-time PCR: Nucleic acids will be extracted using the DSP virus pathogen kit (QIAGEN, Hilden, Germany). The samples will be stored at -80°C until use. A specific HCV test will be performed to confirm active HCV infection (HCV-RNA positive) on a Rotor Gene Q unit (QIAGEN).

PHASE II: SCREENING AT CAÑADA REAL GALIANA

Screening for HCV and the other viruses (HIV, HBV, and HDV) covered by this project will take place at Cañada Real Galiana. Screening will be conducted by nursing personnel hired specifically for that purpose. An active search will be made for

individuals who come into the shantytown from outside, and the detection test will be offered to everyone regardless of whether or not their serological condition is already known. Selection of individuals for screening will be random and consecutive.

Nursing personnel will generally be accompanied by an educator from the HRU. PWID will be offered information on prevention and the importance of early diagnosis and referral to health centers.

Information will then be provided on the goals of the study, and persons who agree to take part will be required to sign an informed consent form, provide contact data for subsequent location, and provide basic social and demographic data and information on their consumption habits. Regardless of whether or not individuals agree to take part in the study, they will all be given a card containing basic information on prevention and contact data for the internal medicine department of HUIL and the HRU.

Data will be recorded on social and demographic details, the consumption habits of each PWID, and the acceptability of the screening test. All data will be recoded in the database by the nurse or the educator using a mobile device with Internet capability and REDCap software. Informed consent forms will be kept in the files of the researcher at the HRU office.

The Whatman™ cards will be stored in a portable refrigerator after processing (see below) and shipped via courier to the National Microbiology Center (CNM) for analysis.

If a test result is positive, the patient will be contacted and offered referral to a specialist health care center in accordance with the study algorithm to confirm the result and assess whether monitoring should begin. All subjects included in the study may contact the medical department or the Harm Reduction Unit directly to make an appointment and collect the results of their tests.

PHASE III: EFFECTIVENESS OF THE INTERVENTION

Evaluation of the effectiveness of the intervention. Drug users who test positive will be contacted and offered the possibility of referral to a specialist health care center, where access to standard confirmation tests used in hospital and access to treatment will be offered. As specified in the consent form, if a patient with a positive result cannot be contacted, regional registries and hospital patient data will be used to find the patient.

Once the patient has been found or contacted, referral to HUIL will be offered to him. The harm reduction team accompanies the drug user to hospital to be evaluated by a practitioner with expertise in assessment of liver disease severity and HCV treatment. In the first visit to the hospital, a part from the medical history and physical examination, a blood test and a Fibroscan® will be performed. These blood tests will confirm the results from the screening for HCV in Cañada Real.

At this point, all PWID will be offered substitution therapy with methadone and referral to a detoxification center. If the patient accepts, he will then be taken to a detox center to start HCV therapy and where he will stay for an average of six months. If the patient does not accept referral to a detox center, the mobile harm reduction unit will administer therapy (directly observed therapy) to him and will be in charge off his follow up in the shantytown.

Hepatitis C therapy will be prescribed according to European and regional guidelines. Current guidelines recommend treating PWID independently of the liver fibrosis stage. In this project, antiviral therapy will be chosen regarding interactions with other drugs or methadone and a once-daily therapy is preferred in those patients who decline being referred to a detox centre.

After HCV therapy has been completed, all PWID will be periodically screened for hepatitis C (analyzing RNA HCV) to detect infection. Depending on the drug users risk factors, screening for reinfection of HCV will be carried out on a 3 to 6 months schedule.

Infanta Leonor Hospital (HUIL) is the reference specialized health centre for Cañada Real shantytown. All PWID diagnosed of HIV and HCV in this shantytown are referred to the HUIL internal medicine department for evaluation. The hospital is highly coordinated with the harm reduction unit. Nurses or doctors from the harm reduction unit accompany the patients to the specialized clinic.

ETHICAL ISSUES

All patients will sign an informed consent form. Confidentiality will be maintained with regard to personal and genetic data in accordance with the basic ethical principles for research involving biological samples and the provisions of applicable legislation (Organic Law 15/1999 of 13 December on Data Protection, Law 41/2002 on Patient and Healthcare Autonomy and Law 14/1986 on General Health). The ethical research principles set out in the Declaration of Helsinki and the Belmont Report will be adhered to, as will those of the UNESCO Universal Declaration on the Human Genome and Human Rights. The requirements of Spanish legislation on processing on personal data will also be met. The research project has been approved by the Research Committee at HUIL and the Ethics Committee of Hospital General Universitario Gregorio Marañón and ISCIII.

LIMITATIONS OF THE STUDY

The study may be limited by the possibility of not reaching the required number of subjects, although we believe this to be unlikely given the large number of people who visit Cañada Real Galiana. Furthermore, the limit of HCV detection by real-time PCR is approximately 1000 IU/mL, although we believe that with this limit we may detect the vast majority of active infections, since HCV viral load in untreated patients tends to be greater than 1000 IU/mL. Moreover, previous publications show 94% sensitivity and specificity for the Whatman card.

In contrast with the rapid tests, sample processing is centralized, so results are not available immediately. Given that screening is performed in an unfavorable setting and on an outpatient basis, a series of problems could arise, as follows: (1) errors in the processing of samples (eg, insufficient sample volume contamination, loss); (2) difficulties in contacting participants once the results are available; and (3) the assumption that although a significant percentage of consumers will consent to screening, many will refuse referral to a health center for confirmation of diagnosis and treatment.

GENERAL TASKS

1. Administrative tasks: Classification by the Spanish Agency for Medicines and Medical Devices (AEMPS) and presentation to the Clinical Research Ethics Committee (CEIC).
2. Contracting of IT services. Design of online data collection forms.
3. Final design of protocol and informed consent forms.
4. Design of database and adaptation of online data collection forms.
5. Initial meeting of researchers.
6. Six-monthly teleconferences for researchers.
7. Phase 1: Recruitment, collection of informed consent forms, collection of

2.4 Study Flowchart

internal validation sample.

8. Phase 1: Refining of screening tests.
9. Phase 1: Processing of samples at ISCIII with extraction of DNA/RNA/antibodies from Whatman cards.
10. Phase 1: Screening tests on controlled samples from patients at HUIL.
11. Phase 1: Analysis of internal validation data.
12. Phase 1: Monitoring and quality control.
13. Phase 1: Reporting of results.
14. Phase 2: Education in prevention and training in screening for healthcare personnel.
15. Phase 2: Recruitment and collection of informed consent forms. Screening at Cañada Real Galiana. Sampling and sending of samples to ISCIII.
16. Phase 2: Processing of samples at ISCIII with extraction of DNA/RNA/antibodies from Whatman cards.
17. Phase 2: Screening tests on samples from subjects at Cañada Real Galiana.
18. Phase 2: Reporting of results to HUIL-Madrid Positivo.
19. Phase 2: Contact with patients who have tested positive.
20. Phase 3: Referral of patients to HUIL.
21. Phase 3: HCV diagnosis & treatment.
22. Phase 3: Follow up after HCV therapy.
23. Phase 3: Monitoring and control of baseline and final quality.
24. Cleansing of the epidemiological/clinical database and cross-referencing with laboratory data.
25. Statistical analysis.
26. Reporting of final results (congresses, papers, doctoral theses).
27. Drafting of the final report.

TASKS

1. HUIL: 1, 2, 3, 4, 5, 6, 7, 12, 19, 20, 21, 22, 23, 24, 25, 26, 27..
2. HRU Madrid Positivo/PH: 3, 4, 5, 6, 14, 15, 19, 20, 21, 22, 23.
3. ISCIII: 5, 6, 8, 9, 10, 11, 12, 13, 16, 17, 18, 23, 24, 25, 26, 27

KEY TO ABBREVIATIONS

AEMPS: Spanish Agency for Medicines and Medical Devices. CEIC: Clinical Research Ethics Committee. HUIL: Hospital Universitario Infanta Leonor (Infanta Leonor Teaching Hospital). ISCIII: Instituto de Salud Carlos III (Carlos III Health Institute).

FIGURE 2.4.1: INTERNAL VALIDATION TESTING AT HUIL

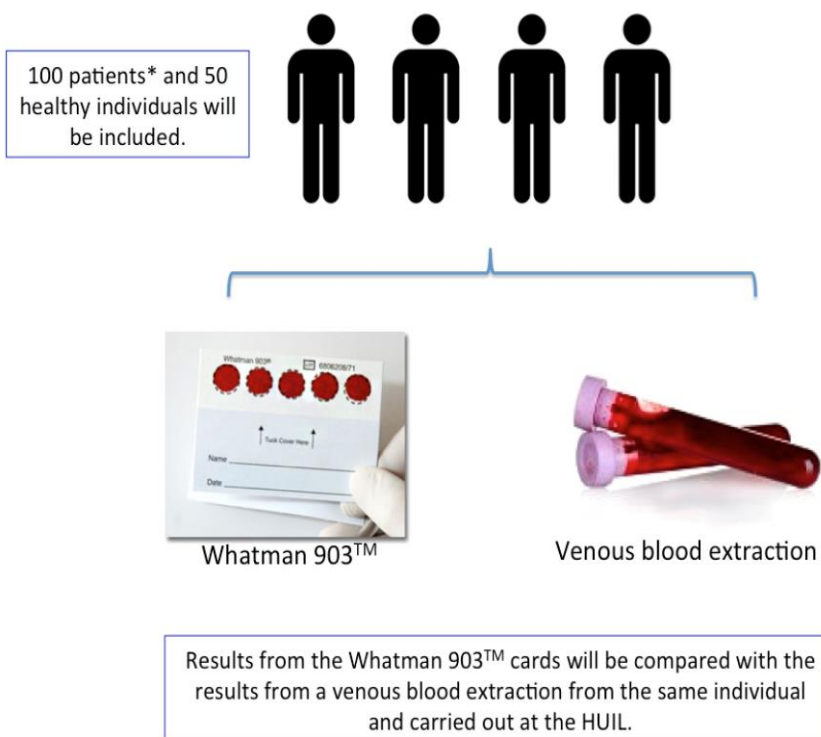


FIGURE 2.4.2: SCREENING IN CAÑADA REAL.

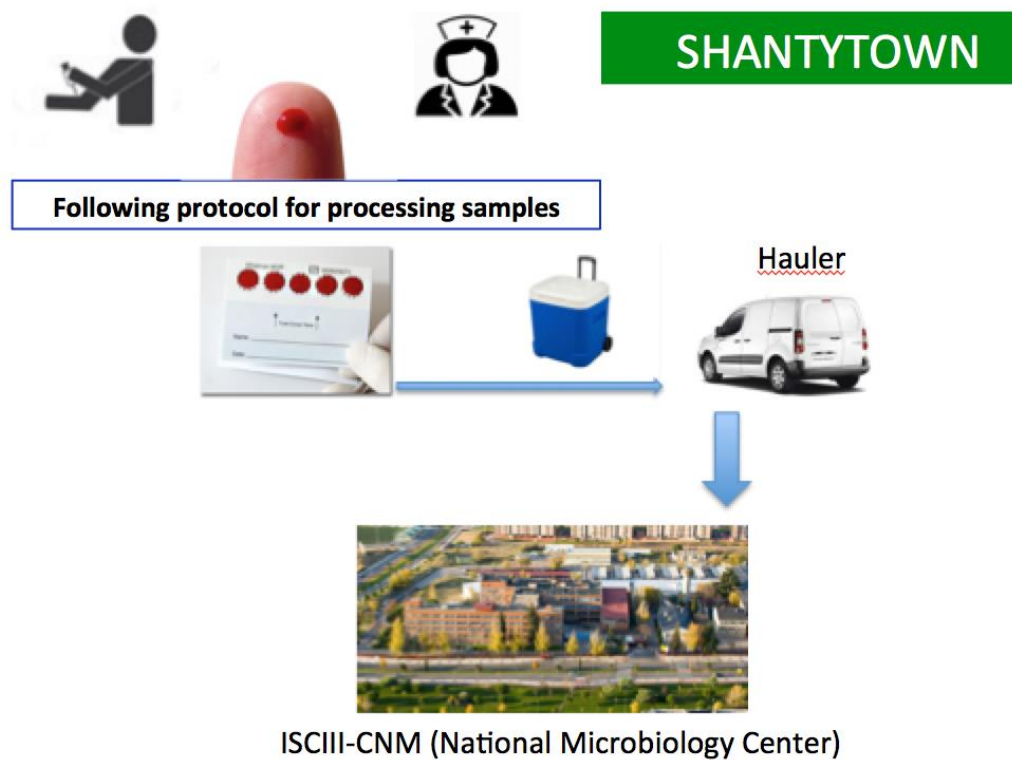


FIGURE 2.4.3: LAB TESTS IN ISCIII

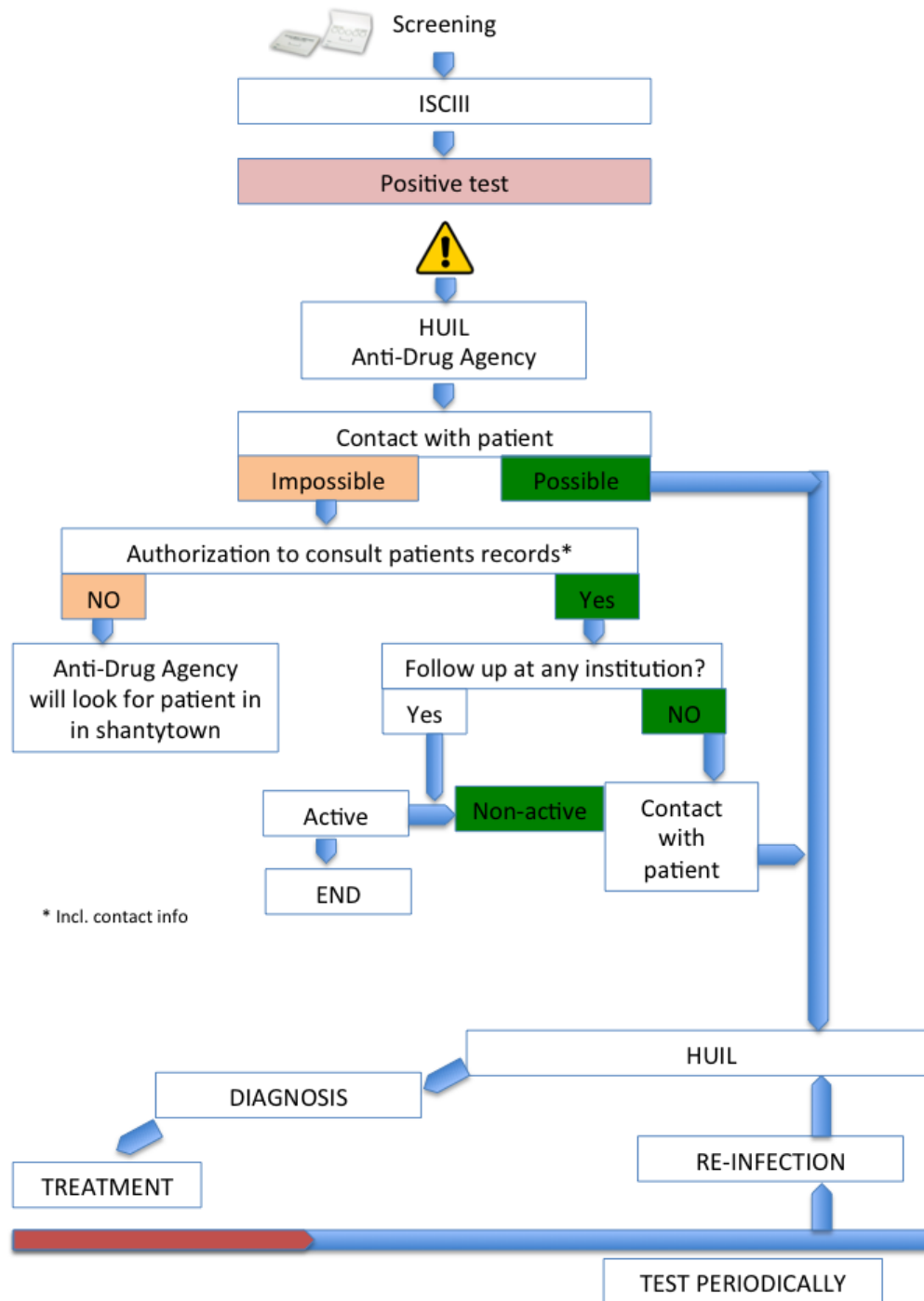
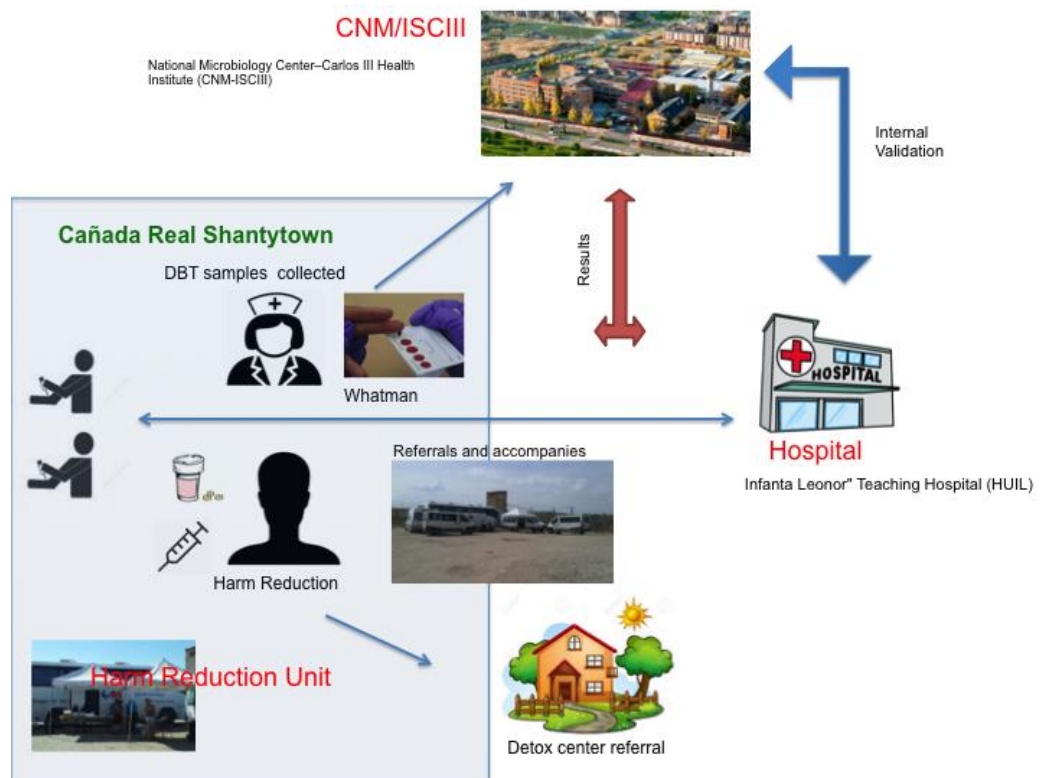
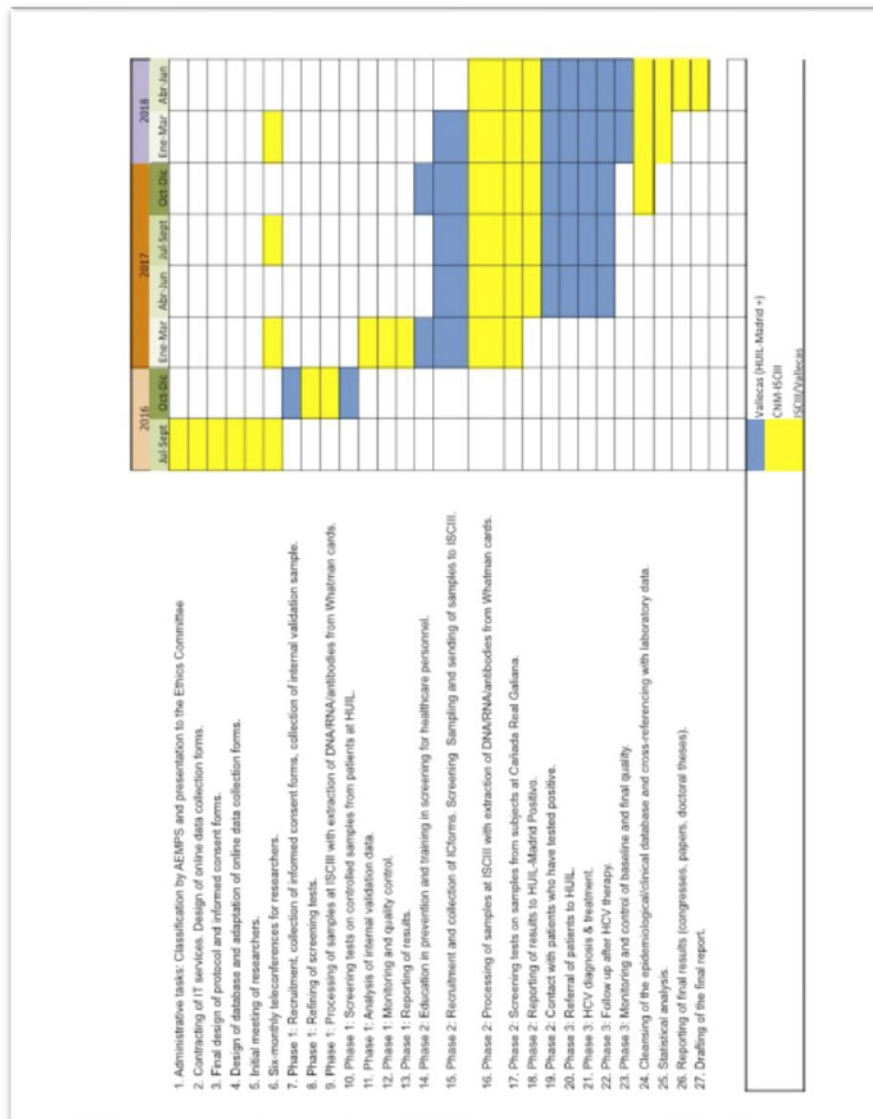


FIGURE 2.4.5: COORDINATION



DURATION OF THE STUDY: 24 months

2.6 Study Duration



2.7 Statistical Analysis and Sample Size Justification

PHASE I

Once all subjects have been included, the results from the Whatman 903 cards will be compared with those obtained by venipuncture at HUIL. Diagnostic performance will be assessed by calculating the area under the ROC curve (AUROC) for each virus. The Se, Sp, PPV, and NPV for each virus will also be calculated.

PHASE II

Sample size: Assuming that around 10,000 people visit Cañada Real Galiana on different days, a sample size of 930 randomly selected subjects will suffice to estimate, with a 95% confidence interval and a precision of ± 3 percentage points, a

population percentage considered to be around 60%. A replacement rate of 0% has been anticipated.

PHASE III

In order to evaluate the effectiveness of the intervention, the accuracy of HCV tests will be calculated at the population level (AUROC, Se, Sp, PPV, and NPV), as will the percentage of patients arriving at the clinic, the percentage of patients treated, and the percentage of patients cured of hepatitis C. The incidence of HCV reinfection will be assessed using a Kaplan-Meier survival method. In addition, the effectiveness of the intervention for HIV, HBV, and HDV infection will be evaluated.

Statistical Methods

The statistical analysis will be performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, New York, USA) and the R statistical package, version 3.2.2 (GNU General Public License). All tests will be two-tailed, with p-values of <0.05 considered statistically significant.

An assessment will be performed to determine the percentage of patients who are positive at screening, are referred to a health center, have their diagnoses confirmed, and are treated at the end of the study. Factors associated with virus transmission, lack of referral to a health center, and HCV treatment will also be analyzed.

Continuous variables will be presented as mean and standard deviation; categorical data will be presented as absolute numbers and percentages. The prevalence (95% confidence interval) of antibody for HCV, HIV, and HDV, HBsAg, and active HCV infection will be calculated in the sample and in the subgroups studied. Categorical data and proportions will be analyzed using the chi-square test or Fisher exact test, as required. The *t* test, ANOVA, or Mann-Whitney test will be used to compare continuous variables. The association study will be performed using logistic regression and adjusting for the major epidemiological and clinical covariates.

VARIABLES INCLUDED

PHASE I: VARIABLES TO BE RECORDED DURING INTERNAL VALIDATION TESTING

- Date of birth
- Gender
- Date of sample collection
- Date of last analysis
- HCV antibody result from last analysis (positive/negative)
- HCV-RNA result from last analysis
- Genotype result from last analysis
- HIV antibody result from last analysis (positive/negative)
- CD4+ lymphocyte count for HIV-positive patients
- RNA-HIV result for HIV-positive patients
- HDV antibody result from last analysis (positive/negative)
- HBsAg result from last analysis (positive/negative)
- DNA-HBV result from last analysis
- Incidents or errors in sample collection (yes/no)

PHASE II: VARIABLES TO BE RECORDED DURING SCREENING

- Code allocated
- Date of sample
- Date of birth
- Gender (male/female)
- Nationality
- Homeless (yes/no)
- No. of days per week that the individual visits Cañada Real Galiana
- Drugs consumed (mark as relevant):
 - Heroin
 - Cocaine
 - Marihuana
 - Alcohol
 - Benzodiazepines
- Current use of parenteral methods (yes/no)
- Date when parenteral methods were first used
- Frequency of injections (daily [No. of times]/weekly [No. of times])
- Syringe sharing for >3 months (yes/no)
- Frequency of syringe sharing (daily/weekly/monthly)
- Date of switch from parenteral methods to smoking
- On methadone program (yes/no)
- Being monitored for HCV infection (yes/no)
- Have you previously been offered the chance to undergo HIV and/or HCV testing?
 - No
 - Yes, but I did not accept
 - Yes, with (positive/negative) results for HIV and (positive/negative) results for HCV
- Contact with health center (never, occasionally, regular monitoring).
 - Primary health care
 - Hospital
 - Drug abuse center
 - Other
- Advantages of alternative screening
- Drawbacks of alternative screening
- Contact data provided (yes/no)
- Post-test contact (yes/no)

PHASE III: VARIABLES TO BE RECORDED AFTER LABORATORY ANALYSIS

- HCV antibody result (positive/negative)
- HCV-RNA result
- Genotype result
- HIV antibody result (positive/negative)
- HIV-RNA result for HIV-positive patients
- HDV antibody result (positive/negative)
- HBsAg result (positive/negative)
- HBV-DNA result
- Incidents or errors in sample collection (yes/no)
- Referred to health center (yes/no)
- HCV antibody result from health center laboratory (positive/negative)
- HCV-RNA result from health center laboratory

	<ul style="list-style-type: none"> • Genotype result from health center laboratory • HIV antibody result from health center laboratory (positive/negative) • HIV-RNA result from health center laboratory • HBsAg result from health center laboratory (positive/negative) • DNA-HBV result from health center laboratory • Treated with antivirals (yes/no) • Grade of liver fibrosis (according to METAVIR or in kPa) • Regimen used for the treatment of HCV • Lost to follow-up (yes/no) • Duration of therapy • Sustained virological response at 12 weeks after finishing therapy (yes/no)
2.11 Reference s	<p>MAIN BIBLIOGRAPHICAL REFERENCES</p> <ol style="list-style-type: none"> 1. OMS Hepatitis C [Internet]. WHO. [citado 23 de abril de 2016]. Recuperado a partir de: http://www.who.int/mediacentre/factsheets/fs164/es/ 2. Gordon SC, Pockros PJ, Terrault NA, Hoop RS, Buikema A, Nerenz D, et al. Hepatol Baltim Md. noviembre de 2012;56(5):1651-60. 3. Lavanchy D. Liver Int Off J Int Assoc Study Liver. enero de 2009;29 Suppl 1:74-81. 4. Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. Lancet Lond Engl. 21 de marzo de 2015;385(9973):1124-35. 5. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Lancet Lond Engl. 15 de diciembre de 2012;380(9859):2095-128. 6. European Association for Study of Liver.. J Hepatol. julio de 2015;63(1):199-236. 7. Eckman MH, Talal AH, Gordon SC, Schiff E, Sherman KE. Clin Infect Dis Off Publ Infect Dis Soc Am. mayo de 2013;56(10):1382-93. 8. Mehta SH, Astemborski J, Kirk GD, Strathdee SA, Nelson KE, Vlahov D, et al. J Infect Dis. 1 de marzo de 2011;203(5):587-94. 9. Maher L, Li J, Jalaludin B, Chant KG, Kaldor JM. Aust N Z J Public Health. febrero de 2007;31(1):30-5. 10. Wiessing L, Ferri M, Grady B, Kantzanou M, Sperle I, Cullen KJ, et al. PloS One. 2014;9(7):e103345. 11. Pouget ER, Hagan H, Des Jarlais DC. Addict Abingdon Engl. junio de 2012;107(6):1057-65. 12. Gowing L, Farrell MF, Bornemann R, Sullivan LE, Ali R. Cochrane Database Syst Rev. 2011;(8):CD004145. 13. Hagan H, Pouget ER, Des Jarlais DC. J Infect Dis. 1 de julio de 2011;204(1):74-83. 14. Turner KME, Hutchinson S, Vickerman P, Hope V, Craine N, Palmateer N, et al. Addict Abingdon Engl. noviembre de 2011;106(11):1978-88. 15. Dore GJ, Ward J, Thursz M. J Viral Hepat. mayo de 2014;21 Suppl 1:1-4. 16. Martin NK, Foster GR, Vilar J, Ryder S, Cramp ME, Gordon F, et al. J Viral Hepat. abril de 2015;22(4):399-408. 17. Edlin BR, Seal KH, Lorvick J, Kral AH, Ciccarone DH, Moore LD, et al. N Engl J Med. 19 de julio de 2001;345(3):211-5. 18. Grebely J, Bryant J, Hull P, Hopwood M, Lavis Y, Dore GJ, et al. J Viral Hepat. abril de 2011;18(4):e104-16. 19. Swan D, Long J, Carr O, Flanagan J, Irish H, Keating S, et al. AIDS Patient Care STDs. diciembre de 2010;24(12):753-62. 20. Ministerio de Sanidad, Servicios Sociales e Igualdad. Plan Estratégico para el Abordaje de la Hepatitis C en el Sistema Nacional de Salud. 21. Wolfram I, Petroff D, Bätz O, Jedrysiak K, Kramer J, Tenckhoff H, et al. J Hepatol. junio de 2015;62(6):1256-64. 22. Saludes V, González V, Planas R, Matas L, Ausina V, Martró E. World J Gastroenterol. 7 de abril de 2014;20(13):3431-42. 23. Edlin BR, Winkelstein ER. Antiviral Res. octubre de 2014;110:79-93. 24. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. J Hepatol. noviembre de 2014;61(1 Suppl):S45-57. 25. Hickman M, McDonald T, Judd A, Nichols T, Hope V, Skidmore S, et al. J Viral Hepat. abril de 2008;15(4):250-4. 26. Craine N, Parry J, O'Toole J, D'Arcy S, Lyons M. J Viral Hepat. marzo de 2009;16(3):219-22. 27. McLeod A, Weir A, Aitken C, Gunson R, Templeton K, Molyneaux P, et al. J Epidemiol

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