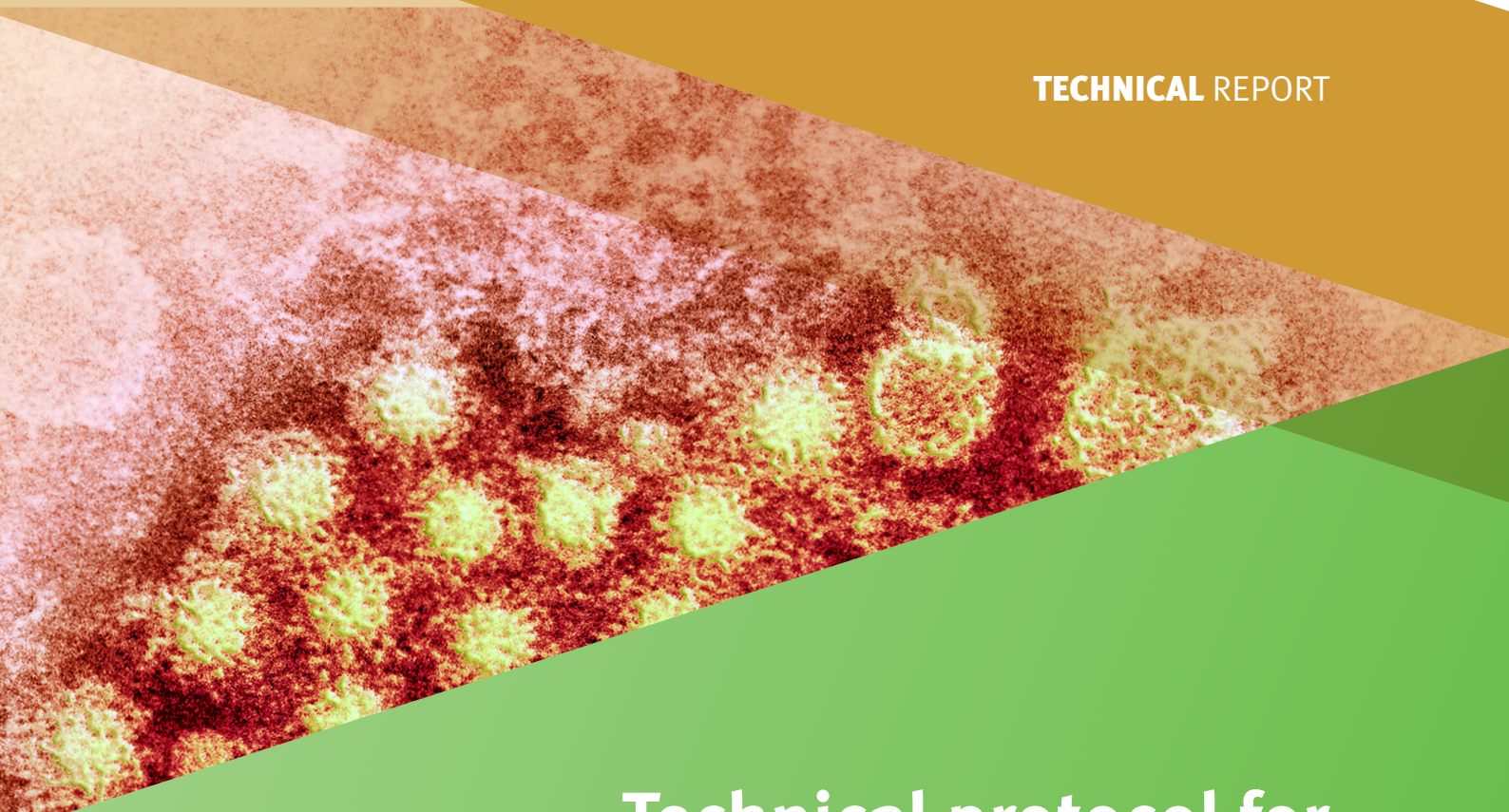


TECHNICAL REPORT



Technical protocol for hepatitis C prevalence surveys in the general population

SPHERE-C Project

ECDC TECHNICAL REPORT

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Abbreviations

HCV	Hepatitis C virus
HCVcAg	HCV core antigen
HES	Health examination survey
LIA	Line immunoassay
MSM	Men who have sex with men
MPES	Multi-parameter evidence synthesis
PWID	People who inject drugs

Introduction

1. Background

Hepatitis C virus (HCV) infection is widely prevalent in most areas of the world. Most acute infections progress to chronic HCV, and HCV is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma [1, 2]. In recent years, highly effective direct-acting antiviral (DAA) treatment options for HCV infection have become available, widely replacing the much less effective and side-effect ridden interferon-based treatments. This new treatment perspective has made the elimination of HCV a feasible target, and so the work towards the elimination of viral hepatitis is gaining momentum in the world. The combat of viral hepatitis was listed as one of the goals of the 2030 Agenda for Sustainable Development, adopted by the United Nations General Assembly in September 2015 [3]; at the same time, WHO published a 'Manual for the development and assessment of national viral hepatitis plans' [4]. Less than a year later, the first 'Global health sector strategy on viral hepatitis 2016–2021' was endorsed by the World Health Assembly, offering a strategy for the health sector on how to work towards the elimination of viral hepatitis as a major public health threat by 2030 [5]. Building upon this strategy, the 'Action plan for the health sector response to viral hepatitis in the WHO European Region' was developed and approved by the WHO Regional Committee in September 2016 [6]. This document is intended to guide and support countries in their efforts to achieve the targets set in the Global Health Sector Strategy, and in 2019 the first progress results were published by the WHO [7].

In both the Global Health Sector Strategy and the European Action Plan it is recognised that an understanding of the HCV epidemic is crucial to inform action. The first strategic direction deals with the need for robust national hepatitis strategic information systems and one of the targets defined for 2020 by the European action plan requires 'Member States to have a national hepatitis infection surveillance program [...] that can detect outbreaks in a timely manner, assess trends in incidence, inform disease burden estimates and effectively track 'in real time' the viral hepatitis diagnosis, treatment and care cascade, including in specific vulnerable groups'.

Most European countries have a surveillance system for HCV in place that is based on routine notifications. EU/EEA countries are requested to upload surveillance data on HCV into the European Surveillance System (TESSy) on a yearly basis, using the EU case definitions. However, recent surveillance data reported to ECDC indicate that a few countries in the EU/EEA experienced difficulties in providing any data on newly diagnosed HCV cases, and that for the majority of countries completeness is a major issue, as well as reporting their data in accordance with EU case definitions [8, 9]. Efforts to synchronise case definitions and differentiate between acute and chronic cases have been made, but have so far not been completely successful. Due to the differences in surveillance systems and the varying case definitions being used, the possibilities to interpret and compare HCV notification data across countries remain limited.

For many infections, the surveillance system is an adequate source to determine incidence, prevalence, burden and trends of disease, and to inform public health decision makers. In the case of HCV, the notification data do not provide a clear epidemiological picture. An important reason for this is the largely asymptomatic nature of the infection, due to which diagnosis is often made many years past the acquisition of the virus, when liver disease has already developed. Surveillance data for hepatitis C thus tend to reflect local testing policies rather than the true incidence or prevalence of the infection. Indeed, countries with extensive screening programmes in key population groups report higher notification rates than others, while at the same time prevalence studies reveal that these countries often have lower prevalence than countries without extensive screening programmes and with lower notification rates [10, 11].

An alternative data source to generate estimates of HCV prevalence is screening of different subpopulations, such as blood donors or pregnant women. In a recent review of the literature performed by ECDC to provide estimates of prevalence of HCV in the general population it was concluded that many countries have data on HCV prevalence levels from screening of these two groups [12]. These data can help inform national estimates of HCV prevalence, but blood donors and pregnant women are not representative of the general population at large. The selection of blood donors is affected by whether blood donation is paid, and the screening results may be further biased through the application of a pre-screening questionnaire on potential risks. HCV prevalence estimates among pregnant women may often be higher compared to the general population, reflecting the fact that the contribution of migrant groups from HCV endemic countries are often underrepresented in general population studies but over-represented among pregnant women [12]. Moreover, estimates in this group may not be representative of the male population where important gender-specific drivers of HCV transmission, such as intravenous drug use and sex between men, have a considerable impact.

A further option to generate estimates of HCV prevalence is prevalence surveys. Surveys, in contrast to surveillance data, provide a snapshot of the current epidemiological situation, as all individuals currently infected are identified, regardless of diagnostic status, if appropriate testing is undertaken as part of the survey. However, as a review by

ECDC found, there is a lack of high-quality, recent, representative, nationwide prevalence surveys, and the methodologies of the available surveys are heterogeneous [12].

2. Rationale

The ECDC strategic direction for hepatitis surveillance is focused on improving the quality of HBV and HCV surveillance data, also by moving beyond notification-based data and working on alternative methodologies for surveillance. ECDC has established an online HCV prevalence studies database (<https://ecdc.europa.eu/en/all-topics-zhepatitis-ctools/hepatitis-c-prevalence-database>) and is developing a larger toolkit for prevalence surveys to support Member States in generating prevalence estimates, in both the general population and key populations, to gain a better understanding of the epidemiology. The toolkit will include a decision algorithm to assist Member States in selecting the type of survey that should be undertaken; it will also provide modelled estimates of the national burden of viral hepatitis.

A reliable estimate of the HCV prevalence in the general population is a fundamental piece of information for the understanding of the current epidemiological situation. This information is important, as it:

- facilitates the appropriate scale-up of prevention and control activities, including treatment, at the national and regional level, in order to plan and prioritise services;
- provides invaluable information for statistical models for disease progression and measuring the impact of prevention and control activities, including treatment;
- constitutes an important reference point for improving the interpretation of other surveillance activities such as routine case based reporting; and
- feeds into the work of generating national estimates, an important indicator which will help reach aims in viral hepatitis strategies.

The prevalence of chronic HCV is also one of ten core indicators (C.1.b), identified by WHO in their framework on monitoring and evaluation for viral hepatitis B and C, which was developed to monitor and evaluate progress towards the goals of the Global Health Sector Strategy at the country level [13]. Having an estimate of chronic HCV is particularly important since DAAs became available. Hepatitis C prevalence in this protocol refers to chronic hepatitis C (anti-HCV and HCV RNA infected) unless otherwise specified.

Prevalence surveys aim to provide representative estimates for the population under investigation. However, methodologies vary widely, as does the quality of estimates in the EU/EEA [12]. The different study designs, sampling strategies and sample sizes make comparisons both within and between countries difficult, and findings can often not be generalised.

The rationale behind the development of an HCV prevalence survey protocol for EU/EEA countries is to establish a minimum standard with regard to methodology in order to generate estimates that are robust and generalisable. By including several protocol standards, ranging from a minimum to higher-level standards, the survey can be performed at different resource levels while, at the same time, optimising scientific output and public health benefits. Furthermore, standardisation will improve comparability between countries.

3. Protocol development

The development of the sero-prevalence surveys of hepatitis C in Europe (SPHERE-C) protocol is based on scientific information, evidence, an inquiry among the hepatitis focal points in the EU/EEA Member States for priorities needed for viral hepatitis, and consultations with experts during three expert meetings. Development also drew on three pilot projects conducted in 2018:

- Bulgaria: a stand-alone survey was piloted in the city of Stara Zagora, Bulgaria
- Finland: a nested survey was piloted; blood samples from the FinHealth2017 study were (retrospectively) tested for HCV
- Italy: a stand-alone survey was tested in the city of Catanzaro, Italy

During the first expert meeting (6–7 December 2016) consensus was reached to focus on the general population, and draft survey objectives were formulated. It was also agreed that probability-based sampling methods should be applied.

During the second expert meeting (18–19 September 2017), the methodology and the planning of pilot projects was discussed. It was decided to highlight how the SPHERE-C protocol fits into the broader context of developing national HCV prevalence estimates. The experts also expressed their interest in alternative methods that yielded good results.

At the third and final expert meeting (2–3 April 2019) the results and lessons learnt from the three pilot projects as well as their implications for the protocol were discussed in detail. This resulted in the final version of the SPHERE-C protocol.

4. How to use the SPHERE-C protocol

The purpose of this protocol is to provide Member States with technical support for the generation of reliable estimates of hepatitis C prevalence in the general population. Three different approaches are proposed, with each approach tied to a specific set of steps. For each methodological step of the protocol, the a) minimum requirements are listed, along with b) the gold standard and c) suggested options for possible expansion of the survey. The minimum requirements refer to criteria that must be met, while the gold standard should still be aimed for. Suggestions on how to further expand the survey are given if sufficient resources are available.

The SPHERE-C protocol consists of two parts:

- Part A: Selection of survey approach and context
- Part B: Planning and conducting the survey; reporting the survey results

Part A provides background knowledge and a guide as to which approach is best in which situation and context.

Part B explains how to perform an HCV prevalence survey.

A tick box at the beginning of each chapter in part B indicates to which of the study approaches the chapter is relevant. The chapters not directly relevant for the selected survey approach may however be relevant as background information and useful to read, regardless of which type of survey approach is chosen. At the end of some chapters, a comment section containing practical considerations (marked in green) has been included.

How often a prevalence survey should be performed depends on the country context (e.g. prevalence level, screening efforts, treatment policy, available resources, etc.), but is also dependent on targets and timelines in national viral hepatitis plans.

Part A. Selection of survey approach and context

1. Objectives

The prevalence surveys of hepatitis C described in this protocol have the following primary objective:

- To estimate the prevalence of chronic HCV infection in the adult general population

The secondary objectives are optional and may be included if resources are available. They are defined as follows:

- To estimate the fraction with chronic, undiagnosed HCV infection in the general population
- To estimate the proportion of past (cleared) infection in the general population
- To estimate the distribution of infection risks among those with past or current HCV infection in the general population

The primary (1.a) and the first secondary (2.a) objectives correspond to indicators C.1b and C.6 of the ten core indicators identified by WHO [13].

Comment: Depending on study approach, further objectives relevant for the local context may be formulated and included in the survey. For example, the survey could be expanded to include additional infections, such as hepatitis B and HIV. This is not covered in any detail in the protocol.

2. Selecting a study approach

Three approaches fulfil the criteria outlined in this protocol, and all three are variations of a probability-based survey design. Please note that surveys are subject to specific local conditions that may preclude the execution of certain survey types.

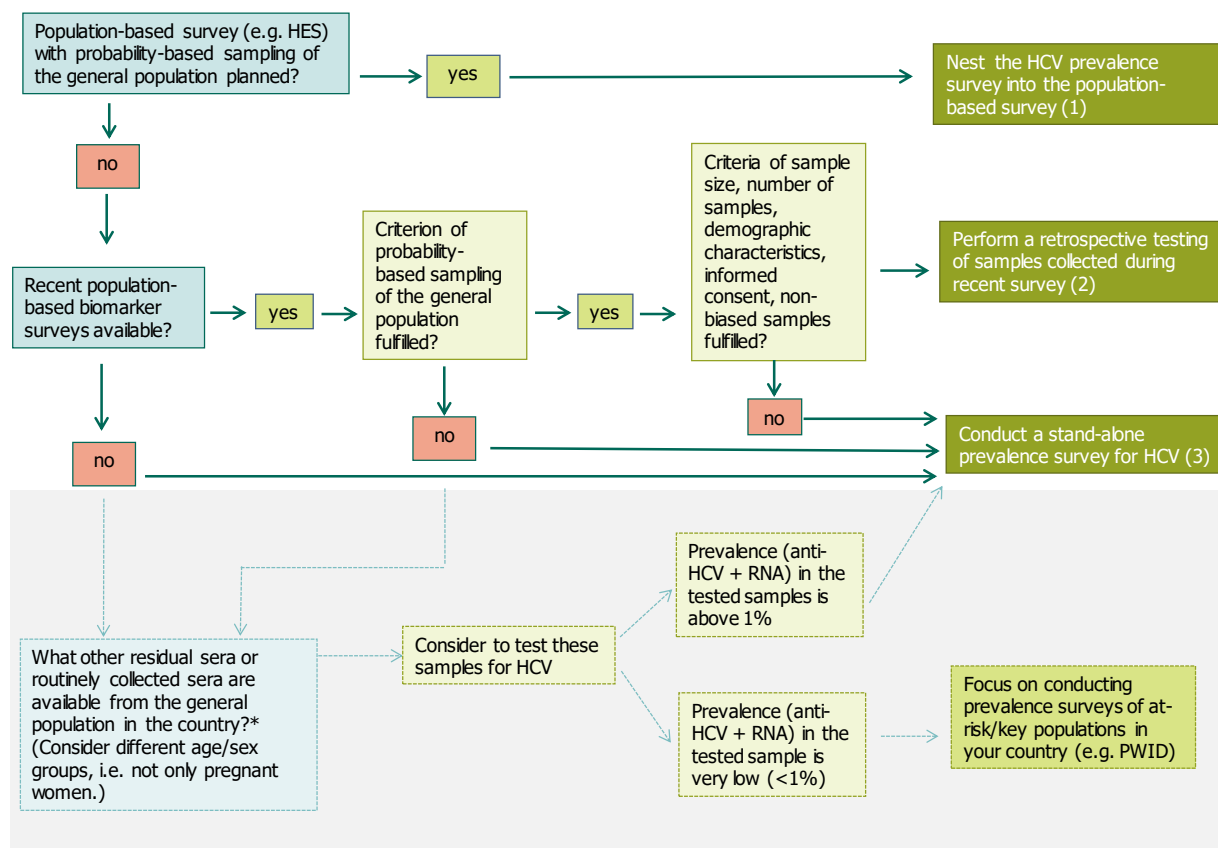
2.1 Points to consider when planning a prevalence survey for hepatitis C

Conducting a national population-based survey will often be a time-, personnel- and cost-intensive exercise – especially if there is no recent or planned population survey. Therefore, countries are advised to follow a careful decision process before choosing an appropriate survey type for estimating hepatitis C prevalence in the general population (Figure 1). If a large population-based survey is planned that includes blood samples – for example a health examination survey (HES) with probability-based sampling of the general population and a sample size sufficient to estimate HCV prevalence – we suggest nesting the HCV prevalence survey into this population survey, provided the planned survey fulfils the minimum criteria outlined in this protocol, e.g. has a sufficiently large sample and is representative of the populations of interest (1). This implies that the survey should be representative at a national and subnational level and that representativeness extends to urban and rural areas. Including HCV testing in existing survey protocols involves steps similar to those for designing a new survey, although some steps may be simpler as they have already been done for the original survey such as ethical approval, sampling process and the recruitment strategy.

If a previous survey (HES or a study with a probability-based sample of the general population) included the collection of blood samples, sera from this survey can be tested retrospectively as long as the above-mentioned criteria are met. The number of available blood specimens should be investigated because the number of residual sera available for testing may be much lower than the overall number of participants, which would preclude this approach (2). Researchers should take into account whether the residual samples may be biased in terms of either age or disease status before selecting this type of survey.

If the two options above are not available, a stand-alone survey with the sole purpose of estimating the HCV prevalence should be conducted. When conducting a stand-alone survey, researchers cannot skip any steps and need to design the survey from scratch (3).

As illustrated in Figure 1 (grey box), countries should consider reviewing available materials and data to get an idea of the national HCV epidemic before embarking on a new stand-alone HCV prevalence survey. Researchers are advised to look for alternative residual sera of the general population – even those from routine procedures – which, as a first step, could be used for HCV testing (see Chapter 3 for examples).

Figure 1. Points to be considered when planning a prevalence survey for hepatitis C in the general population

* Alternative options exist that might be explored by countries to get an idea of the HCV prevalence level in the general population, if they do not have data from a recent population-based prevalence survey or plans for a future survey and few resources for a stand-alone survey. These are further described in Chapter 3.

2.2 Collection through nested surveys

An HCV prevalence survey in the general population may be done by nesting a survey within an existing larger health survey of the general population, e.g. a HES or a national population survey with a different focus. The concept of nesting is to make use of an already planned survey and benefit directly from this larger survey because participants can be tested for HCV with little effort.

This requires that the main survey also targets the general population and aims for a sufficiently large sample (see Chapter 1 (Study population) and Chapter 4 on sample size). An important advantage of nesting the HCV prevalence survey within a larger population-based health examination survey is that these surveys have a rigorous sampling strategy to ensure a representative sample and low non-response rates. Nesting an HCV survey within a large-scale survey will generate a robust prevalence estimate while requiring relatively few resources. This may require close coordination across survey components. A list of planned HESs in EU/EEA countries can be found in Appendix 1.

The total planning time needed for a nested survey is dependent on the planning and implementation of the larger survey. Ideally, the HCV survey coordinator is also part of the health survey planning team. Even if all goes well (e.g. data protection and ethical issues for the nested HCV survey are resolved), additional time needs to be scheduled to develop an HCV-specific study protocol, test for HCV markers in the laboratory, analyse and report the results, and inform participants of the test results.

Strengths of collection through nested surveys

- Makes use of an already established survey tool (sampling approach already developed)
- If nested within a HES, socio-demographic and behavioural data are usually collected
- Usually performed on a regular basis if part of a HES
- Requires fewer resources
- May support exploration of comorbidities and, possibly, more distant outcomes through linkage to mortality or other data if the main survey supports such linkages

Weaknesses of collection through nested surveys

- Limited possibilities to influence the sampling strategy
- Data collection depends on the schedule of the survey
- Limited possibility to change questions if key questions or covariates of interest to HCV are missing

2.3 Retrospective testing

Blood specimens collected during a probability-based survey of the general population can be used for an HCV prevalence survey because residual specimens can be tested retrospectively for HCV. This implies that a HES or another probability-based survey of the general population was performed and that blood specimens were collected and are (still) available for further testing; in addition, participants should have given their consent for further use of the samples.

Prior to testing, it should be explored whether target population, sample size and other details of the population-based survey meet the criteria described in the HCV protocol as presented in this document. The number of available blood specimens should be ascertained because the number of residual sera samples may be lower than the overall number of participants, which could preclude this approach. Whether the leftover samples are a biased subset of the original set also needs to be considered because this may compromise the validity of the HCV prevalence estimate. For example, the original survey may have focussed on sera from older people, and the only sera left over are from younger people. Ideally, samples should not be older than five years, but exceptions can be made depending on the country context (e.g. treatment strategy). An overview of national HESs performed in, or planned for, 2000–2022 in EU/EEA countries can be found in Appendix 1.

Another possibility is to retrospectively test residual specimens from a former HES or other probability-based survey of the general population through unlinked anonymous testing. This is in some ways more straight forward as the aim is to estimate the HCV prevalence rather than to diagnose patients and with unlinked anonymous testing the results are not given back to the participants. Before testing, every specimen is irreversibly unlinked from information that would be linked to a specific individual. This approach has to be included in the informed consent of the original survey; the additional criteria mentioned above should also be met.

Retrospective surveys tend to be very efficient because the time invested is limited to the period of sample extraction and testing – provided that all ethical aspects were already taken care of and that samples were easily accessible.

Advantages of retrospective testing

- Simple to conduct
- Not time-consuming
- Inexpensive

Disadvantages of retrospective testing

- Administration of a questionnaire not possible
- Sampling strategy cannot be influenced
- Requires that a population-based survey was performed and that blood specimens are available
- If samples were stored over an extended period of time, sample extraction and testing may be difficult (e.g. RNA)
- In order to reflect the current epidemiology, samples should not be older than five years, but this also depends on the country context (for example, point in time when treatment scale-up was initiated)
- No influence on timeline and dependent on the timeline of the HES
- Residual sera may be a biased subset

2.4 Stand-alone survey

The main purpose of an ad hoc stand-alone survey is to measure HCV prevalence. A stand-alone survey is independent of the availability of a HES or other surveys and can be performed in any country. Stand-alone surveys require the planning and execution of all steps as described in the below protocol and are therefore relatively resource-intensive. On the other hand, stand-alone surveys allow for a high degree of customisation to exactly suit the locally agreed objectives.

Advantages of stand-alone surveys

- Optimal to target the selected population
- Administration of questionnaires possible
- Do not require a planned or recent HES

Disadvantages of stand-alone surveys

- Time-consuming
- More costly
- Require more human resources than other survey types

A stand-alone survey is a very time-consuming approach because of the numerous planning steps (e.g. identifying a sampling strategy, accessing a suitable sampling frame, receiving ethical and data protection clearance, and implementation). The amount of time needed also depends on factors such as, for example, previous experience of the project team, available infrastructure for the survey (e.g. testing of samples) and the number of staff members.

Comment: In countries with a very low expected prevalence of HCV in the general population, the sample size needs to be very large; researchers have to weigh the benefits of a precise estimate versus the invested efforts and expenses of conducting a stand-alone survey.

3. SPHERE-C project in context

The SPHERE-C protocol focuses on measuring disease prevalence in the general population. Knowing the HCV prevalence in the general population, however, is only one element of estimating national HCV prevalence. In order to get a national HCV prevalence estimate, additional activities beyond what is covered in the SPHERE-C protocol may be needed. The aim of this chapter is to place the SPHERE-C project in a wider context and briefly address some of the key aspects which countries need to be aware of when planning an HCV prevalence survey. This chapter covers the following aspects:

- Moving from HCV prevalence surveys in the general population to national HCV prevalence estimates
- Alternative methods that may be considered if probability-based sampling is not possible/affordable

3.1 From HCV prevalence surveys in the general population to national HCV prevalence estimates

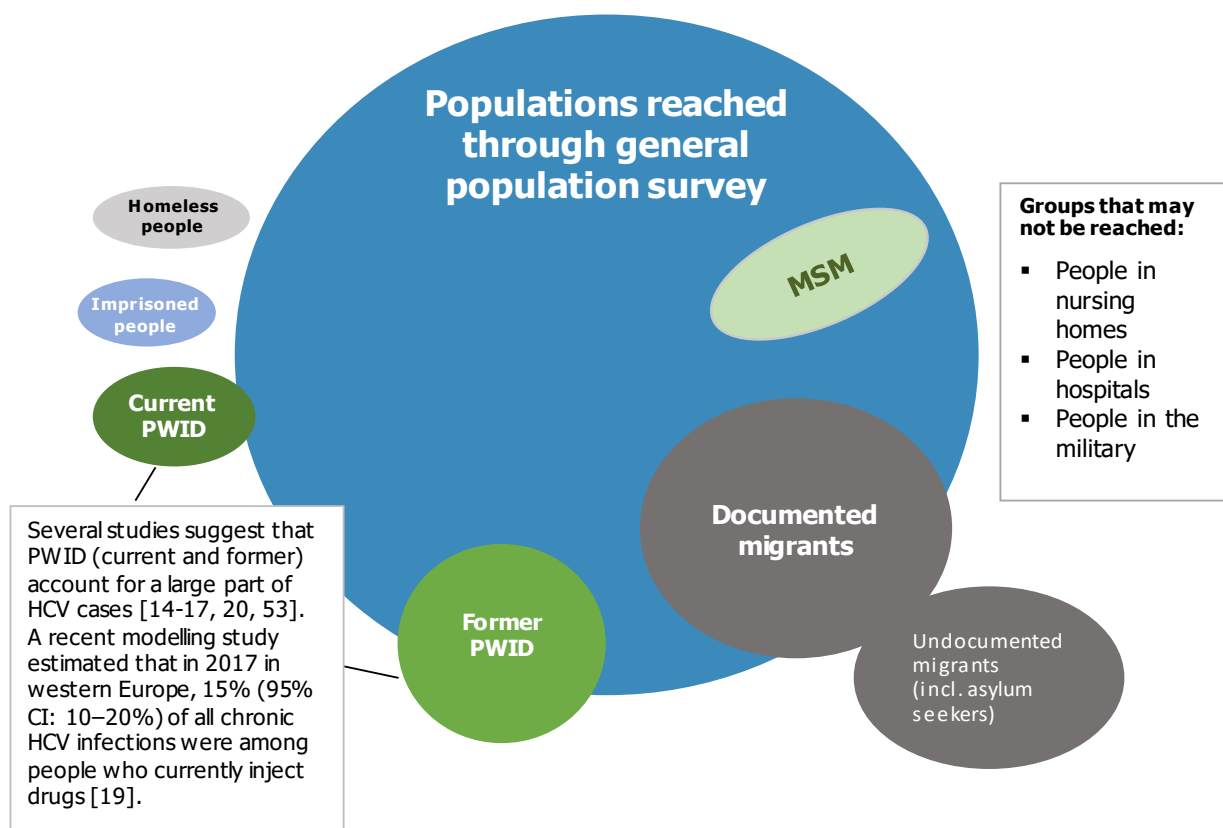
Additional activities needed to collect data for estimating national HCV prevalence include the following:

- Estimations of population sizes for key populations at risk of HCV. For many European countries, PWID (people who inject drugs) are the most important risk group, and estimates of population size should include both former and active PWID. Also important are size estimates of the prison population, the MSM (men who have sex with men) population and the migrant population (documented and undocumented).
- Smaller surveys and regular monitoring to measure HCV prevalence in these subgroups. Data on active PWID may be garnered through low-threshold drug services such as needle-exchange surveys, drug treatment services (e.g. opioid substitution therapy), drug consumption rooms, and prisons.

It is important to consider to what extent populations not captured through general population surveys contribute to the total burden/prevalence of HCV. Modelling studies from the United Kingdom and the United States suggest that the majority of people living with chronic hepatitis C are either active or former PWID, with 'never injectors' contributing substantially less to the total burden of HCV (estimates from the UK suggest around 15%) [14-18]. However, the epidemiology varies across Europe, and in some EU countries, iatrogenic transmission and non-PWID-related transmission among migrants and MSM also play an important role [18, 19].

Figure 2 illustrates the estimated relative size of the key populations most at risk for hepatitis C and to what extent these may be reached through a general population survey. The relative size of subpopulations (i.e. active and former PWID, documented and undocumented migrants, prisoners, homeless people, MSM, hospitalised people, and people in nursing homes) and the proportion likely to be reached through a general population survey will vary from country to country. People can belong to several risk groups (e.g. migrants and MSM and PWID), but Figure 2, for illustration purposes, assigns individuals belonging to several risk groups only to the risk group with the highest risk of HCV (e.g. PWID).

Figure 2. Overview of key populations at increased risk of HCV and to what extent these might be reached by a general population survey



Note: The size of risk groups and how well they can be reached through a general population survey varies at the national and subnational levels.

The population groups with the highest burden of HCV are often difficult to reach. This is a serious limitation when estimating national HCV prevalence through general population surveys. In 2015, it was estimated that the US National Health and Nutrition Examination Survey (NHANES) missed about 30% of chronic HCV cases [15]; this percentage was reduced to approximately 11% for the 2013–2016 estimates [20]. Another important issue affecting representativeness and completeness of information is non-response (see also Chapter 6 on recruitment strategies). The response rates observed in several recent HES [21-24], e.g. from Finland, Germany, the Netherlands and the US (NHANES), were at around 50%. This shows the importance of estimating the population sizes of key populations at risk of HCV. Additional surveys are needed to measure HCV prevalence within risk groups.

3.2 Alternative approaches

As mentioned above, there are alternative options for countries that want to explore HCV prevalence levels in the general population but neither have data from recent population-based prevalence surveys nor plans for a future survey. Many alternative options are based on non-probability-based methods that increase the risk of bias: HCV prevalence estimates based on this study type must be interpreted with caution.

3.2.1 Residual sera from clinical laboratory samples

Ireland: Residual sera from the national reference laboratory (N=3 795 specimens tested)

In 2016, the Health Protection Surveillance Centre in Ireland undertook a survey of residual sera from the national virus reference laboratory in order to estimate HCV prevalence among the adult population in Ireland [25]. In Ireland, the National Virus Reference Laboratory provides diagnostic and reference services for clinicians investigating viral infections throughout the whole country; around 200 000 blood specimens (equal to 150 000 serum specimens) are received annually for diagnostic purposes, antenatal screening, and pre-employment screening. In the 2016 study, the following steps were taken to reduce potential bias: a) to minimise bias by age group and sex, the sampling frame was stratified before sampling and sampled with probability proportional to the size of the strata in the general population. In order to adjust for geographical bias in sample selection and under-sampling in three age/sex strata, the analysis was weighted for geographical area, age group and sex; b) specimens from STI clinics and drug treatment services as well as specimens submitted specifically for HCV testing were excluded to avoid an overestimation of the HCV prevalence in the general population. In total, 53 specimens (1.4%) were seropositive (anti-HCV and HCV Ag), and age-sex-area weighted seroprevalence was 0.98%.

For countries with national reference laboratories this option may be a quick and low-cost way to obtain (non-probability-based) population estimates of HCV prevalence which can give a good idea of the level of prevalence to be expected in the general population, if efforts are made to minimise bias (the potential for bias is possibly quite large). The estimated costs of the Irish study were EUR 35 000. Staffing costs were covered by the institutions involved in the project.

Belgium: Residual sera from 28 laboratories (N=3209 specimens tested)

In a published study from Belgium [26], 28 clinical laboratories collected 3 209 specimens from surgery, orthopaedic, emergency and otorhinolaryngology wards. An age-stratified sample size was calculated based on the recommendations of the European Sero-Epidemiology Network (ESEN) project [27], which implied oversampling in the age group below 20 years (54% of specimens collected) because the study used residual samples from a serum bank to obtain seroprevalence estimates for multiple diseases, including vaccine-preventable diseases, to obtain precise estimates for younger age groups. Residual sera were collected between July 2013 and January 2015. To avoid oversampling ill or susceptible populations, laboratories were asked to exclude specimens from immunocompromised patients, patients from intensive care units, and those with evidence of multiple blood transfusions; laboratories did this, but inconsistently. Information on age, sex and district was collected for each specimen. Nine laboratories received specimens from correctional facilities, and one from a needle exchange programme (six laboratories did not provide information on this). In total, 14 specimens (0.44%) were ELISA positive, eight were positive or indeterminate by line immunoassay (LIA) and tested by RT-qPCR. In total, four had chronic HCV, two had resolved the infection, and for two the HCV status remained indeterminate. The results were weighted for sex, age and population by district and adjusted for cluster sampling, which resulted in 0.26% (95% CI 0.10–0.64%) HCV seropositivity and 0.13% (95% CI 0.04–0.43%) chronic HCV prevalence among those older than 20 years. This was very similar to the estimated HCV prevalence in the Netherlands [28].

Romania: Residual sera from 145 hospital laboratories (N=3 266 specimens tested)

In Romania, prospective collection of leftover sera from 145 hospital laboratories was carried out between September and November 2013. Systematic sampling by county, age group and sex was done to generate a sample representative of the hospitalised population in Romania. The following exclusion criteria were used: patients admitted to infectious diseases hospitals/wards; patients with liver-biliary-pancreatic medical/surgical pathology as these were assumed to have an increased risk of HCV; also excluded were samples from blood transfusion centres because donors were considered to be healthier than the general population [29]. This study found an anti-HCV prevalence of 5.6%, higher than the 3.2% prevalence found in the general population survey conducted in Romania in 2006–2008 [30]. This indicates that despite using clear exclusion criteria, using leftover samples from hospitalised patients may lead to an overestimation of prevalence in the general population.

3.2.2 First-time blood donors

Most countries in the EU/EEA have recent data on HCV prevalence among first-time blood donors [31, 32]. These data can be used to give a first indication of the lower limits of the prevalence of HCV, especially if compared with similar data from other EU/EEA countries. However, comparison across countries can be difficult due to differences in the blood donor pre-selection criteria and whether blood donations are paid or voluntary. In most countries, first-time blood donors are not representative of the general population.

3.2.3 Pregnant women

A recent systematic review on HCV prevalence carried out by ECDC [12] identified estimates of HCV prevalence among pregnant women in eight EU/EEA Member States (Austria, Greece, Ireland, Italy, the Netherlands, Norway, Slovenia and Spain). A study from Slovenia used unlinked anonymous testing on residual sera obtained for routine syphilis screening from more than 30 000 pregnant women [33]. Similar to blood donor data, data on HCV prevalence among pregnant women may have strong bias and thus may not represent the general population. In countries with high levels of inward migration, HCV prevalence in pregnant women may be higher if compared to all women in the general population. This reflects the fact that migrant groups from HCV-endemic countries tend to be overrepresented among pregnant women as compared to the general population [12]. Moreover, estimates among pregnant women will not be representative of the male population where important gender-specific drivers of HCV transmission, such as intravenous drug use and sex between men, have a considerable impact.

3.2.4 Emergency units

Recently, several European countries conducted HCV surveys in selected urban emergency departments [34–37]. As the population attending hospital emergency departments often has an increased risk of HCV, these surveys often detect a higher HCV prevalence than what would be expected compared with the general population. The cited examples from Frankfurt and Berlin (Germany), Dublin (Ireland) and London (UK) found an HCV antibody prevalence of 1.6%–5.1%, indicating an HCV prevalence level of up to more than five times higher than for the general population [34, 36, 37].

3.2.5 General practitioner (GP) or health insurance registries

Several studies included in ECDC's systematic review on HCV prevalence [12] under the heading 'general population' were conducted among people recruited through 'primary healthcare insurance units' [38] or general practitioner (GP) records [39-44]. A large variance was found in how participants were recruited, ranging from convenience sampling to randomised sampling. In countries where registries from GPs, primary healthcare units or national healthcare insurances cover a large proportion (>75%) of the general population, registries may well be used as sampling frames to study the general population. It is, however, very important to keep in mind which part of the population is not covered by the sampling frame and then take into account the bias introduced should this population have a different risk of HCV.

Large studies have been conducted in both France and Poland, using a multistage sampling design of either the population registered in the primary health care units [45] or beneficiaries of the national health insurance system [38]. In both studies the sampling frame was expected to cover around 80% of the general population. The French study included participants between 18 and 80 years of age. The Polish study included participants aged 18 years and above and excluded those currently on treatment for HCV. Both the French and Polish studies are examples of well-planned designs that limit potential bias and produce reliable prevalence estimates for the general population.

3.2.6 Patients attending free preventive medical examinations

Patients attending a medical check-up can easily be screened for HCV; results were used as a proxy for the general population in studies performed in Croatia, Germany and Spain [46-48].

The German study [47] included 51 GP practices and performed HCV testing on a convenience sample of individuals coming for a free medical check-up ('35+ check-up') offered by the German health insurance scheme. Participants also filled in a questionnaire with information on risk behaviours. In total, just over 21 000 people were included in this study. Anti-HCV prevalence was 0.95% – three times higher than in the German general population survey [23].

3.2.7 Other convenience populations (military and pre-employment screening)

One populations that may fairly easily be screened for HCV would be military personnel [49]. It is also possible to combine HCV testing with pre-employment screenings. Both approaches do not give a representative picture of the prevalence in the general population.

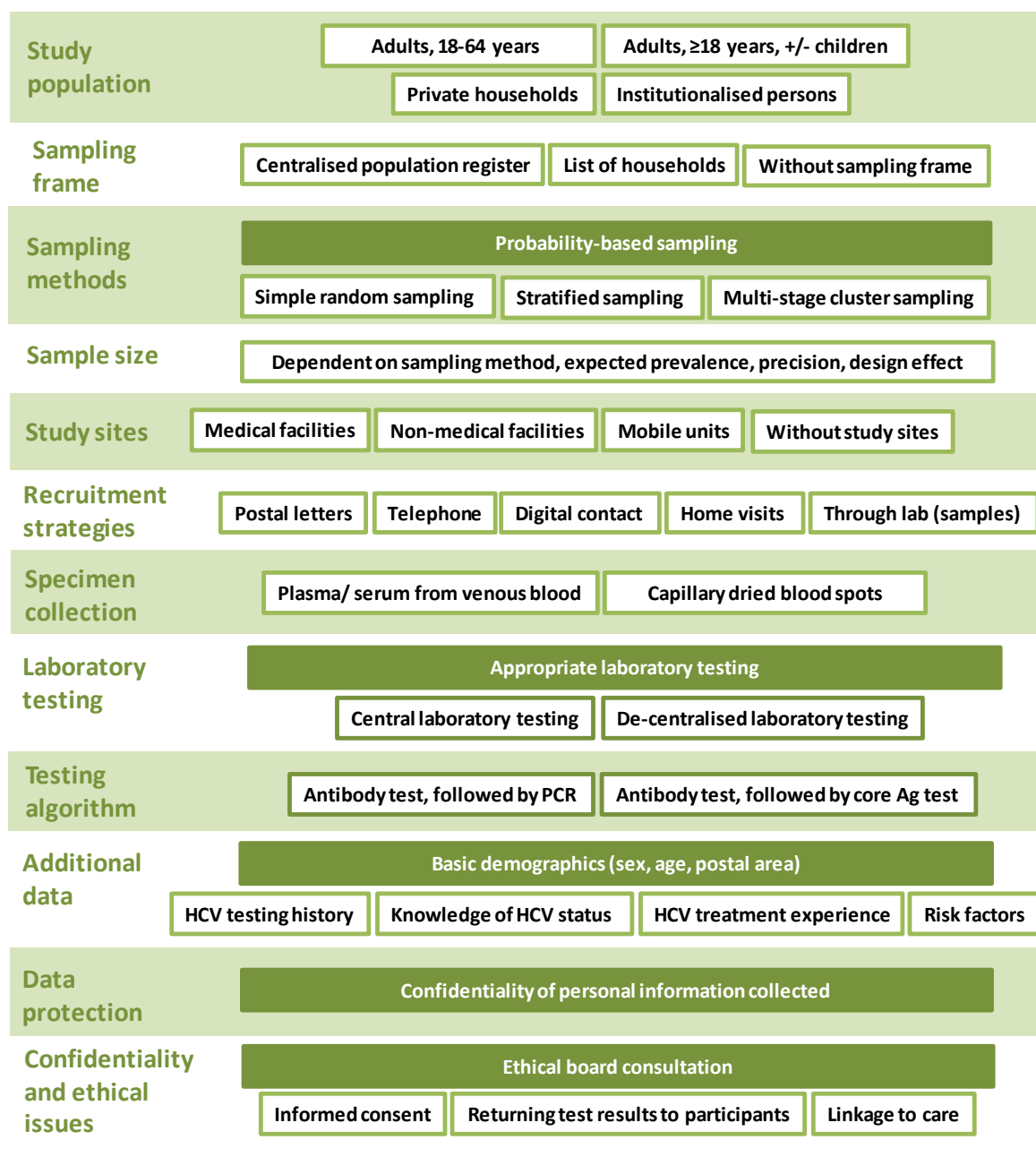
3.2.8 Modelling approaches to estimate national chronic HCV prevalence

In Europe, various modelling approaches have been used to estimate national HCV prevalence. One study used a relatively simple epidemiological model that took into account mortality, HCV incidence and diagnosis rates and applied them to the 2004 French seroprevalence survey [38] to estimate the size of the population with undiagnosed chronic HCV infection in France in 2014 [50]. In another study in the Netherlands, the national anti-HCV prevalence was estimated both for 2011 and 2016 by combining population size estimates and HCV prevalence estimates for up to nine high- and low-risk population groups, using data from multiple sources [28, 51]. In Denmark, researchers used information from several national registries collecting information on people diagnosed with hepatitis C. They identified overlap between registry entries, using the nation-wide unique personal identifier and employing capture–recapture analysis and other assumptions to estimate chronic HCV prevalence in Denmark [52]. In England, researchers have been estimating chronic HCV prevalence in England and Wales using an MPES approach (multi-parameter evidence synthesis) for over a decade [14, 16, 53]. The latest national estimates for England combined data on severe HCV-related liver disease and disease progression by using an MPES approach; in addition, the number of infections among PWID and data on diagnosis and treatment were used to reconstruct historical HCV incidence and estimate the chronic HCV prevalence in 2015 [54]. In recent years, a number of global modelling studies were conducted addressing viral hepatitis prevalence [55, 56]. ECDC is currently working on applying MPES methodology to develop national HCV prevalence estimates for all EU/EEA countries.

Part B. Planning and conducting the survey; reporting the survey results

This part contains practical information for conducting an HCV prevalence survey. An overview of the components of the protocol can be found in Figure 3.

Figure 3. Overview of mandatory requirements and methodological options for an HCV prevalence survey



Legend:

Mandatory requirements

Methodological options

1. Study population

Nested survey collection Residual sera testing Stand-alone survey

The population targeted by this protocol is the adult general population. The study population should at a minimum be defined as all persons ≥ 18 years of age who are resident in a private household in a Member State, regardless of citizenship or language. Eligibility criteria for participation in the survey should not exclude risk groups, especially not people who inject (or have previously injected) drugs.

Table 1. Definition of study population

	Minimum requirement	Possible expansion
Age	≥ 18 years	≥ 18 years ¹
Sex	Males and females	-
Type of housing	Private households	Institutions ²
Coverage	Subnational ³	National

¹May also include children and/or adolescents if justified by notification data or other data sources

²E.g. nursing homes, retirement homes, military barracks, etc. Not included are homeless shelters, prisons and other detention facilities.

³E.g. regional or city level.

It is important to consider that the proportion of institutionalised individuals (e.g. in elderly homes) increases with age, making sampling and recruitment more challenging in older age groups. The age limit may be lowered to include younger age groups if local HCV notification data imply significant transmission among children and adolescents. It should be kept in mind that including minors in the study population may require additional attention to ethical issues, such as consent from a legal guardian. As the expected prevalence in this subpopulation is likely to be lower than among the adult population, this should be taken into account when calculating sample size.

Residence data may be expanded to include institutions in addition to private households. Institutions could include nursing homes, elderly residential homes or military barracks. Although homeless shelters, prisons and other detention facilities, such as juvenile detention facilities, jails, etc. also count as institutions, including them in the survey is not recommended. As prisoners and the homeless population count as high-risk groups for HCV, a survey with different objectives and methodology is needed for estimating prevalence in these populations. Including institutionalised individuals in the study population will improve the completeness of the epidemiological picture, but may require significant additional resources if sampling frames for these populations have to be constructed. Depending on the type of institution, other modes of contact initiation than for participants living in private households may be required.

Comment: In order to generate a prevalence estimate for the general population in the country, a survey with a nationally representative sample is strongly recommended. However, should the available resources be insufficient to conduct a national survey or achieve a nationally representative sample of the population, the survey may have to be reduced to subnational coverage (i.e. one or a few representative regions). This should be considered only in countries where prevalence estimates do not exist or are of very poor quality. The results of a regional survey cannot be simply generalised to the entire country; instead, it should be viewed as an estimate of the sampled region.

2. Sampling frame

Nested survey collection Residual sera testing Stand-alone survey

A sampling frame is a list that covers the entire study population. It is essential for the generation of a probability-based sample.

2.1 List of individuals

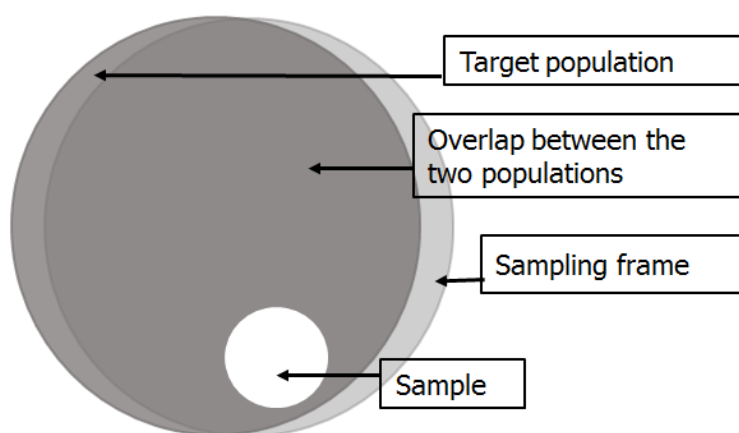
The gold standard for drawing a sampling frame is a comprehensive official person register covering the population of interest. Most EU/EEA Member States maintain a centralised population register (see Appendix 2), but their accessibility for sampling purposes varies between Member States. In the European context, statistical offices and universities are usually granted access to population registers, while commercial survey agencies have more limited access [57]. This should be considered if the survey field work is to be assigned to a commercial agency. If more than one relevant register exists, it is advisable to choose the one that is most frequently updated, because researching outdated contact information may require a lot of resources. Further, specific information about what the sampling frame entails and also what information is available should be researched early during the planning process.

2.2 List of households

If population registers are unavailable, lists of households or addresses may be used as sampling frames. Using a non-person-based register as sampling frame will require an additional sampling stage where individuals are sampled from the selected households or addresses. In England, the postal code register is used as sampling frame for the annual health survey [58]. Some European countries have used 'health user registries' (e.g. Portugal) [59], registers of general practitioners, or public health insurance registers as sampling frames for general population surveys (e.g. Luxembourg) [60]. These lists could also serve as a sampling frame, but it is important to assess which parts of the population might not be captured by the sampling frame and what bias this might introduce to the HCV prevalence estimate.

The sampling frame should cover the population under study as accurately as possible. The register of choice should thus be the most complete and up-to-date listing of the population of interest, though in most sampling frames certain elements will to some degree be incorrectly included or omitted, in addition to duplicates and factual errors, as illustrated in Figure 4. The choice of a suitable frame will be affected by the quality and types of potential sampling frames available in the country. It is advisable to seek advice from a sampling statistician and those with a good knowledge of available sampling frames in the local setting.

Figure 4. Target population and sampling frame in survey sampling



Source: <http://www.theanalysisfactor.com/target-population-sampling-frame/>

Population registers in EU/EEA countries, as described by Poulain and Herm (2013), can be found in Appendix 2 [61]. A thorough overview of sampling frames used in various European surveys is available through the SERISS project¹ and can serve as a point of reference for each Member State interested in drawing a sample from the population:

www.seriss.eu/resources/deliverables

WP2: Representing the population

D2.1 – Report on the use of sampling frames in European studies

Annex 2 (separate Excel document)

Table 2. Requirements for the sampling frame

	Minimum requirement	Gold standard
Sampling frame	List of addresses or households	List of individuals

Comment: A sampling frame is also required if data are collected through nested surveys. If this is the case, suitable sampling frames will be identified by the researchers in charge of the main survey. If retrospective testing is planned, researchers need to ensure that the sampling frame used for the original survey meets their needs and requirements.

¹ SERISS (Synergies for Europe’s Research Infrastructures in the Social Sciences): <http://seriss.eu/>

Tips from SPHERE-C pilot projects

- It is crucial to have a clear understanding of the sampling frame used, i.e. researchers need to know its structure and who is in charge of updating its content. To get this information, publications that used the same sampling frame can be consulted. In Bulgaria, for example, people are registered with a 'permanent' and a 'current' address. The 'permanent address' is the primary address available when the first ID card, which is rarely changed, was issued. The 'current address' is the address where the person currently lives. To make sure that people in the sample could be reached, only those with a 'current address' in Stara Zagora were invited to the survey.
- Keeping the delay between drawing the sample and sending the invitation letters as short as possible is advised to avoid status changes (e.g. change of address, death, etc.).

3. Sampling methods

Nested survey collection

Residual sera testing

Stand-alone survey

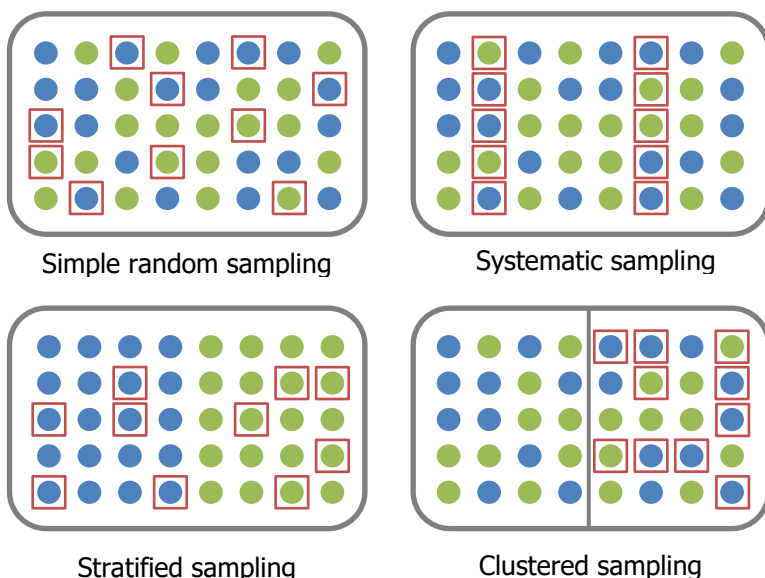
The sample should be selected by using a probability-based random sampling method. This entails that each individual has a known probability of being sampled. The techniques commonly used to draw a probability-based sample are simple random, systematic, cluster and stratified sampling (Figure 5), and these are often combined into multistage sampling procedures. Drawing a probability-based sample may often be a complex procedure; it is strongly recommended that a sampling statistician assists with this step.

Simple random sampling refers to when individuals in the sample frame are selected at random and every individual has the same chance of being in the sample. For this approach, a sampling frame listing all individuals is needed. Simple random sampling is applied for smaller geographical areas, e.g. if a prevalence survey in one city is planned.

Systematic sampling implies that the selection of individuals for the samples is carried out systematically, e.g. every *n*th person on a list is selected (e.g. every 5th, 10th, 100th). No sampling frame is necessary, but some kind of order is required, based, for example, on time, geography, or lists.

Stratified sampling and multi-stage cluster sampling are explained in more detail below.

Figure 5. Probability-based sampling methods



3.1 Stratified sampling

Stratified sampling ensures that the sample is representative of the target population in terms of the characteristics by which it has been stratified. This is an appropriate approach when HCV prevalence is expected to be different in different subgroups in the population, e.g. age groups or sex. This is also important for variables like age that easily introduce a selection bias due to different participation rates. Since all other characteristics of the sample are still chosen by simple random sampling, the total sample size remains the same.

If the expected prevalence differs between the strata of the target population, it might be useful to perform sample size calculation by stratum: for each stratum, the expected prevalence and required precision have to be formulated. In addition, the power for a comparison of the prevalence between different strata should be considered. For a given total sample size, the precision inside the strata is lower than the precision of the estimate for the total prevalence.

Stratified sampling is also useful if different strata require different sampling or recruitment approaches, or if over-sampling of certain strata is desired, which depends on country context and needs. All strata should be sampled.

In order to ensure that the list of the population is as accurate and up-to-date as possible, it is important that the time between drawing the list and sending the invitation letters is as short as possible. It is recommended that the final list is checked by an epidemiologist to ensure that the people in the sample list are randomly selected.

Tips from SPHERE-C pilot projects

During the pilot in Stara Zagora, Bulgaria, the sample – drawn from the population register, ESGRAON² – was stratified by age and sex. The first list, however, was drawn so that only people born every 10th year were included, e.g. 1950, 1960, 1970, and hence needed to be re-drawn. Lists should be checked by a statistician or qualified epidemiologist to ensure that the people included are truly drawn randomly.

3.2 Multi-stage cluster sampling

For stand-alone HCV prevalence surveys, a two-stage cluster sampling design is often the most appropriate approach [62]. In this approach, the country is first divided into small, non-overlapping geographical areas known as clusters or primary sampling units (PSU). These should not be bigger than the area that one study site can cover, i.e. the travel distance to the study site must be feasible for all eligible individuals living within the area of the cluster. The number of eligible individuals living in each PSU must be known and thus small census tracts, municipalities, electoral districts and post code areas are all suitable as PSUs. Before PSUs are sampled, it is good practice to stratify the PSUs by grouping together relatively similar PSUs, e.g. urban versus rural PSUs, PSUs with similar age and sex distribution. This should be done by taking into account the PSU's social and demographic profile – if available in the sampling frame (i.e. the sampling frame has to be a list of individuals, as opposed to addresses or households). A good stratification of the PSUs will help increase precision of survey estimates.

A primary purpose of stratification is to improve the precision of the survey estimates and ensure that the sample adequately represents the population. For this, the formation of the strata should be done so that PSUs in the same stratum are as homogeneous as possible; PSUs in different strata should also be as heterogeneous as possible with respect to the characteristics of interest to the survey.

In the first sampling stage of this design, clusters are selected, preferably using probability proportional to size (PPS). With PPS the probability of a PSU to be selected is made dependent on its size – the larger the PSU the higher its probability to be selected. In the second sampling stage, an equal number of individuals, addresses or households, denoted as secondary sampling units (SSU), are randomly selected with equal probability from each PSU. An important advantage of cluster sampling is that it can significantly reduce costs and resources. By selecting participants in geographical clusters (PSUs) the number of required study sites equals the number of clusters, whereas, had the participants been randomly distributed across the country, more study sites would have been required to maintain a short travel distance for all participants. Another advantage of this sampling strategy is that it only requires lists of all elements in the selected PSUs, rather than of the entire population.

The choice of different sampling methods, such as stratification and cluster sampling, influence the precision of the generated estimates. This is called the design effect, which is defined as the ratio of the variance of a statistic with a complex sample design to the variance of a statistic with a simple random sample design. A larger design effect results in a larger error, and in order to compensate for this, a larger sample size, in comparison to simple random sampling, will be required to achieve the same level of precision (see also Table 3 and Table 4).

3.3 Sampling with non-person based sampling frame

If a non-person-based sampling frame is used, a third sampling stage in which individuals are sampled from the selected household or address will be required. To do this, recruiters need to visit households to select individuals for the sample. This can be done using the Kish grid or the last/next birthday method. The aim of these methods is to avoid introducing a systematic selection bias when selecting participants from households consisting of more than one person.

² More details on the local population register can be found in: 'Integrated information system for demographic statistics ESGRAON-TDS in Bulgaria'; available from: <https://www.demogr.mpg.de/papers/working/wp-2002-010.pdf>

3.3.1 The Kish grid method

The Kish grid method requires that the age and sex of all household members is collected prior to the selection. The grid offers an algorithm which randomly selects one household member [63]. The main advantage of this method is that the selection occurs truly at random and cannot be influenced by the interviewer. A weakness of this method is that the selection process is relatively time-consuming and may therefore result in relatively high dropout rates. However, once the selection process is completed, the dropout rates are relatively low.

3.3.2 The birthday method

The last or next birthday method selects the household member with the most recent or next birthday for participation. The main strength of this method is its quick and straight-forward application. However, since the probability of inclusion will vary with the number of eligible household members, this information must be recorded through the questionnaire in order to take these probabilities into account when calculating the weights (see Chapter 12 for more information about weights). A drawback of this method is the fact that birthdays are not equally distributed over the year, which will introduce a certain bias. Furthermore, this method is not as reliable as the Kish grid because the selection process is the result of a negotiation between the interviewer and the contact person.

Further technical guidance on sampling methods is available from:

- EHES: Manual part A – Planning and preparation of the survey (<http://www.julkari.fi/handle/10024/131502>) [62]
- Levy PD, Lemeshow S. Sampling of populations. Methods and applications. 4th ed. 2008 [64].

Comment: If a survey is only conducted in a relatively small geographical area, e.g. a city or town, simple random sampling or single-stage stratified sampling may be the best approach. If the survey is a nested survey with retrospective testing of residual sera from a larger survey, any sampling design developed for the main survey is acceptable, as long as the definition of the target population corresponds with the one defined for the HCV survey, provided that probability-based methods are applied. If only a subsample of the samples collected for the larger survey is used for the HCV prevalence survey, samples should be selected using stratified sampling (by age, sex and geographical region).

4. Sample size

Nested survey collection

Residual sera testing

Stand-alone survey

Sample size calculation is an important part of the survey and determines the statistical precision of the HCV prevalence estimate. This chapter focuses on how to compute the total sample size.

A national survey would in most cases be conducted as a cluster-randomised survey, whereas a pilot survey in a single region or city would consist of a simple random sample. In cluster-randomised studies, the sample size has to be enlarged due to the so-called design effect, because two participants in the same cluster are usually more similar to each other than two random participants. Preferably, a sample should consist of several small clusters rather than a few large clusters.

For a health examination survey, a minimum sample size of 4 000 is recommended to ensure representativeness, assuming a 70% response rate [62].

The minimum aim for the survey is to generate an overall prevalence estimate. If HCV prevalence is expected to substantially vary between different subgroups of the population, a stratified sample should be chosen. In this case, the survey would generate prevalence estimates for several strata based on age, sex, region, ethnic group, etc. The sample size of a stratified sample is the sum of the sample sizes required for each of the strata. In any case, the expected prevalence should be based on currently available estimates from previous surveys.

The required sample size depends on the following factors:

- The expected prevalence (p_0)
- The alternative prevalence (p_1); this equals the higher or lower bound of the aimed confidence interval
- The power ($1 - \beta$), usually set to 80% or 90%
- The desired confidence level of the estimate (α), usually set to 5% corresponding to a 95% confidence interval
- The expected design effect (d), for simple random sampling $d = 1$, for cluster-randomised sampling $d = 2$ is frequently used (expect variance to be twice as big compared with simple random sampling)
- The expected response rate (r).

The formula used to calculate the sample size is:

$$N = \frac{d}{r} \left(\frac{z_{1-\alpha/2} \sqrt{p_0(1-p_0)} + z_{1-\beta} \sqrt{p_1(1-p_1)}}{p_1 - p_0} \right)^2,$$

where z_x is the x -quantile of the standard normal distribution, e.g. $z_{97.5\%} = 1.96$ (corresponding to a confidence level of 95%). The sample size increases proportionally to the design effect d . The denominator includes the response rate (r), which means that the lower the anticipated response rate, the larger the sample size has to be. In the denominator you also find the difference between the expected and the alternative prevalence $\delta = p_1 - p_0$, also called the target precision of the estimate. This precision has a substantial impact on sample size: the more precise we want the estimate to be, the larger the sample size has to be. In some cases, an alternative formula [65] for the sample size calculation is used. This formula is similar to the formula presented above, but drops the second summand in the numerator, the one starting with $z_{1-\beta}$. This corresponds to a power of 50%, since $z_{50\%} = 0$. However, we would advise to choose a larger power, e.g. 80%. Another modification is the ‘finite population correction’, which is only relevant for very small populations, i.e. when the total population is approximately as big (or smaller) as the sample itself and does not need to be taken into account for a national survey.

Some examples of estimated sample sizes with different levels of expected prevalence and precision can be found in Table 3 (cluster-randomised samples) and Table 4 (simple random sample). The relative benefit of a higher precision and higher number of participants increases if the response rate is high. However, if the expected response rate is low, it is better to invest one’s resources to increase the response rate rather than the sample size [62].

Calculating the sample size in Stata is possible using the one-sample proportion test. Three input parameters are needed: the expected prevalence, the higher or lower alternative prevalence, and the power (usually set to 80%). Alpha (significance) level is set to 5% by default. In addition, design effect and response rate have to be taken into account.

When the expected HCV prevalence is compared to both a higher and lower alternative prevalence, the final sample size should be the maximum sample size calculated for these estimates.

Before nesting an HCV survey into a larger survey or before retrospectively testing residual sera, it should be assessed whether the planned (or achieved) sample size is compatible with the target precision for the HCV prevalence estimate.

Further information on sample size calculation for multiple stage sampling design is available from: <http://www.ehes.info/rc/tools/tools.htm>.

Table 3. Estimated sample size based on different levels of expected prevalence and precision levels (cluster-randomised sampling)

Cluster randomised sampling				
Expected chronic HCV prevalence	Higher or lower alternative HCV prevalence	Estimated net sample size (participants)	Estimated gross sample size	Expected number of HCV positive samples*
0.5%	1.0%	3 942	7 884	20
1.0%	1.5%	7 072	14 144	70
1.0%	2.0%	1 958	3 916	20
2.0%	3.0%	3 494	6 988	70
2.0%	1.0%	2 566	5 132	50
5.0%	7.0%	2 062	4 124	103
5.0%	3.0%	1 630	3 260	82

Settings: design effect: 2.0; confidence level: 95%; response rate: 50%.

Calculated with Stata’s power command.

* Calculated as ‘expected prevalence’ x ‘expected number of participants’

Table 4. Estimated sample size based on different levels of expected prevalence and precision levels (simple random sampling)

Expected chronic HCV prevalence	Higher or lower alternative HCV prevalence	Simple random sampling		
		Estimated net sample size (participants)	Estimated gross sample size	Expected number of HCV positive samples*
0.5%	1.0%	1 971	3 942	10
1.0%	1.5%	3 536	7 072	35
1.0%	2.0%	979	1 958	10
2.0%	3.0%	1 747	3 494	35
2.0%	1.0%	1 283	2 566	25
5.0%	7.0%	1 031	2 062	51
5.0%	3.0%	815	1 630	41

Settings: design effect: 1.0; confidence level: 95%; response rate: 50%

All calculations based on a 95% confidence level; calculated with Stata

* Calculated as 'expected prevalence' x 'expected number of participants'

Tips from SPHERE-C pilot projects

Bulgaria

- Simple random sampling was used to calculate the sample size; expected chronic HCV prevalence was set to 1%; lower alternative prevalence was set to 0.25%
- The following Stata command was used: `power oneproportion .01 .0025, power(.8)`
- A sample size of 999 was calculated; 1 998 people were invited, expecting a response rate of 50%.

Italy

- Stratified random sampling was used to calculate the sample size; expected chronic HCV prevalence was set to 1% in age group 35–65 years; higher alternative prevalence was set to 2.20% and to 5% for the 65+ age group; higher alternative prevalence was 10.0%
- The following Stata commands were used: `power oneproportion .01.022, power(.8)` (age group 35–65) and `power oneproportion .05 .1, power(.8)` (age group 65+)
- Sample sizes were calculated at 704 (age group 35–65) and 185 (age group 65+); total sample size was 889.

5. Selection of study sites

Nested survey collection

Residual sera testing

Stand-alone survey

The sites used for data and specimen collection can be set up in a variety of locations. One option is to set up study sites within the premises of medical facilities such as local health centres or general practitioners (GP) offices. Using medical facilities is particularly helpful when collecting biological samples. Other options include city halls, sports clubs, non-governmental organisations or similar. Study sites can also be combined.

It is also possible to visit participants at home, but this tends to be costly and time-consuming. Study sites should be convenient for the participants in terms of location, accessibility and transportation infrastructure. Study site selection also depends on sociocultural preferences. Pre-survey focus groups are a good means to find out what will work best. Alternatively, experiences from previous surveys could be assessed.

Study site	Strengths	Weaknesses
Health facilities (local health centres, GPs, hospitals, etc.)	<ul style="list-style-type: none"> • Usually have an established system for collection, storage and testing of biological samples • Facilities known to participants and associated with health-related issues • Usually easily reached 	<ul style="list-style-type: none"> • Some participants may not like to visit health facilities
Central public location (e.g. city hall or similar)	<ul style="list-style-type: none"> • Usually easily reached 	<ul style="list-style-type: none"> • Organising the collection, storage and testing of samples requires more effort
Mobile unit/home visits	<ul style="list-style-type: none"> • Making home visits or offering people to be tested close to their home in a mobile unit may increase participation rates 	<ul style="list-style-type: none"> • Requires more time and resources

6. Recruitment strategies

Nested survey collection

Residual sera testing

Stand-alone survey

Potential participants can be contacted by letter, email, telephone, text messages or through a home visit. Social media can be used as well [66]. It is suggested that an invitation letter be used for initial contact, which may be followed up with further letters, postcards, phone calls, electronic reminders (e-mail, text messages or other digital services, including tools supplied by the national/local 'electronic government' infrastructure or the national health insurance system) or home visits. Invitation letters can be sent by normal or registered mail. Sending letters with registered post often involves participants having to pick up the letter at the post office (which may reduce participation rates in some countries if only registered mail is used). One of the advantages of sending letters by registered mail is that it can provide useful information about completeness of the sampling frame (i.e. if people no longer live at the address) and provides verification that a letter was delivered or that a delivery attempt was made.

6.1 Ways to increase participation

Efforts should be made to keep non-response at a minimum because non-response rates above 30% seriously reduce the representativeness of the sample [62].

Many factors have an impact on participation, for example the way invitees are contacted, the degree of 'participation burden' (e.g. how and where data are collected, extent of physical examination, time to complete the questionnaire, participation time, transportation, and cost to reach the study site), promotional activities surrounding the survey, incentives, and options regarding location and schedule [67, 68]. For a nested survey, participation needs to be secured by the team in charge of the main population-based survey, e.g. the HES team. It is essential to determine ahead of time – and together with the team of the main survey – the amount of blood required to accommodate both surveys.

6.1.1 Awareness raising and promotion of the survey

An awareness-raising campaign can help create awareness of the importance of the survey and thereby willingness to take part. There are different ways of disseminating information about a survey, often depending on the country and the context. Options include press releases, press conferences, and the distribution of posters and flyers. Social media are a functional, socially valid communication form, particularly when aiming for younger age groups.

6.1.2 Recruitment of participants

How to invite participants depends on country and context. The recommended number of contact attempts is three (e.g. initial contact by letter; two follow-up attempts, for example letter and phone call).

Recruitment can be through:

- letter (translated into all languages relevant in a setting)
- telephone
- text messages (SMS)
- mobile phone messaging services, e.g. WhatsApp, Snapchat or Threema
- personal visits.

6.1.3 Incentives

Material incentives may also be used, such as reimbursement of travel costs, travel vouchers, small gifts, or the assurance that participation in the survey may qualify as an approved paid day off from work. All incentives have to receive clearance from the local ethics committee.

Unconditional incentives (sent with the invitation letter and independent of participation) have a stronger impact than conditional incentives (provided upon participation).

The type of incentive should be appropriate for the setting (e.g. country, culture and age group). In some countries, monetary incentives work well, in other countries they are frowned upon and perhaps not even acceptable due to ethical considerations. In Finland, providing a personal 'health profile' that allowed participants to compare their profiles to the anonymised profiles of other participants in the same age group was seen as an incentive by many participants. Distance to the study site seems to be a bigger factor in metropolitan areas than in the countryside.

Before selecting a recruitment strategies, a qualitative pre-survey assessment should be conducted, for example by holding focus group discussions to identify efficient recruitment strategies that are acceptable to the vast majority of potential participants. Pre-survey assessment can be done for different population groups, e.g. different age groups or different ethnic groups.

Tips from SPHERE-C pilot projects

- In Bulgaria, a press conference was held on day one of the survey. This worked well in terms of raising awareness for the survey.
- Many registered letters were not picked up in Bulgaria, as these mailings are often associated with bad news. This highlighted the importance of checking what works best in a particular context through pre-survey focus groups
- In Finland, providing a personal 'health profile' that allowed participants to compare their profiles to the anonymised profiles of other participants in the same age group was seen as an incentive by many participants.

Best practice example: recruitment in Greece

In Greece, an HCV survey was nested within the Greek HES. Special recruitment efforts were made to reach Roma and migrants. Recruiting participants through home visits worked well. A person from the local authorities accompanied the interviewer during home visits. More information about the Greek HCV survey can be found here: www.hprolipsis.gr [69].

Further reading:

- Tolonen H. EHES Manual. Part A. Planning and preparation of the survey. 2nd edition. Helsinki: National Institute for Health and Welfare; 2016.
- Topolovec-Vranic J, Natarajan K. The use of social media in recruitment for medical research studies: a scoping review. *Journal of Medical Internet Research*. 2016;18(11):e286.
- Tolonen H, Lundqvist A, Jääskeläinen T, Koskinen S, Koponen P. Reasons for non-participation and ways to enhance participation in health examination surveys – the Health 2011 Survey. *European journal of public health*. 2017;17(5):Pages 909–11.
- Tolonen H, Ahonen S, Jentoft S, Kuulasmaa K, Heldal J. Differences in participation rates and lessons learned about recruitment of participants – the European Health Examination Survey Pilot Project. *Scand J Public Health*. 2015 Mar;43(2):212-9.

7. Specimen collection, storage and transport

Nested survey collection Residual sera testing Stand-alone survey

7.1 Types of specimens

7.1.1 Venous blood samples

Venepuncture samples are the gold standard for the diagnosis of HCV infection. One venous blood sample is sufficient to conduct several serological and molecular tests with high sensitivity; additional testing (e.g. genotyping, resistance testing, viral load, further pathogens) may also be possible.

Venous blood samples offer a faster turnaround time in the laboratory compared to dried blood spots, but they need to be processed (centrifuged) to obtain plasma or serum within a relatively short timeframe. This requires a centrifuge, refrigerated storage, and transportation, all of which are generally accessible in medical centres, but if specimen collection takes place in study sites, the availability of equipment and trained healthcare personnel should be checked first.

7.1.2 Dried blood spots

If a survey is conducted at a site without proper equipment to take venous whole blood specimens or if samples are collected during home visits or through self-collection by the participants, the use of dried blood spot (DBS) specimens can be considered.

DBS sampling is a simple procedure and may even be used for self-sampling, although there may be local regulations that would need to be considered. The capillary blood dots are spotted on filter cards. Blood is applied to pre-punched discs to prepare dried blood spots. DBS turnaround times are longer, due to a specified laboratory testing protocol and pre-processing [70].

Table 5. Type of specimen

	Minimum requirement	Gold standard
Type of specimen	Dried blood spots	Venous blood samples

Comment: Oral fluid testing is not recommended due to a lower antibody concentration in oral fluid compared to blood, which results in reduced sensitivity.

7.2 Collection and processing of specimens

7.2.1 Venous blood samples

Blood must be collected aseptically with disposable syringes and needles and drawn into labelled, sterile vials (tubes). Depending on the country, trained and accredited healthcare personnel (or trained non-medical staff) are required to perform the venepuncture in accordance with professional standards. In order to ensure proper hygienic handling and to avoid accidental injuries or possible transmission of infections to healthcare personnel, it is necessary to disinfect the local area and ensure appropriate use of gloves and the safe disposal of sharps in a needle disposal container. The quantity of blood to be drawn depends on the number of analyses to be performed (usually about 1 mL of serum for each test, e.g. a tube of 10 mL of blood to recover 5 mL of serum). At least a quantity of 3-5 ml should be taken.

The following tubes are to be used:

- Serum separator tube (for serology)
- EDTA tubes (for nucleic acid testing).

Further technical guidance on equipment, centrifuging and drawing samples, including staff training and quality control measures is available from:

- EHES manual part B, Chapter 6.1.1 Blood sample collection [71]
- World Health Organization. WHO Guidelines on drawing blood: best practices in phlebotomy [Internet]. Bethesda, Maryland: NCBI Bookshelf; 2010. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK138665/> [72]

Advantages of testing serum collected by venepuncture

- Venepuncture is the established gold standard
- Provides a standardised approach
- Allows collection of a sufficient amount of blood that permits laboratory testing with more than one assay
- Full range of serological markers possible at high sensitivity and specificity
- Molecular testing possible, including quantitative PCR
- Testing for co-infections possible (if subjects have consented)
- Testing of residual sera possible

Disadvantages of testing serum collected by venepuncture

- Need of appropriately trained (medical) staff
- Logistically challenging: requires adequate transport, storage and processing
- More expensive
- Hygiene and infection control: risks need to be considered
- Invasive procedure: phlebotomy might be poorly accepted and/or difficult to perform in people with poor veins.

7.2.2 Dried blood spots

Collection of dried blood spots (DBS) requires minimal staff training, even non-medical trained staff can collect samples; self-sampling is also possible. The best locations for collecting capillary blood samples for DBS are the 3rd and 4th fingers of the non-dominant hand. After gently massaging the finger to increase blood flow and disinfecting the skin, a skin puncture just off the centre of the finger pad is made, preferably with a retractable lancet to avoid finger-stick injury of the staff. Alternatively, a finger puncture device can be used. The first drop of blood should be wiped away, the next hanging drop of blood is applied to the filter paper. Pre-punched filter papers are commercially available [70]. For different tests, it is recommended that several punches in the filter are filled. To insure a sufficient quantity of blood for testing, blood spots need to be visible on the front and back of the filter paper.

Whole blood is later eluted from the filter paper and used for the test procedure. This comprises the generation of punches from the centre of the circles, the elution of the punches in a phosphate-buffered, saline-based buffer (or other buffer which suits subsequent testing), the recovery of the eluates, and finally centrifugation of the laboratory cups to free the DBS eluates from any debris that may have originated during the elution process. The DBS eluates are then ready for analyses, e.g. through a fully automated platform.

Further technical guidance on how to collect and process DBS is available here:

- World Health Organization. Module 14: Blood collection and handling – DBS. Geneva: WHO; 2005. Available from: https://www.who.int/diagnostics_laboratory/documents/guidance/module14_blood_collection_&_handling_dbs.pdf [73]
- PATH, RBP-EIA: Collecting, processing, and handling venous, capillary, and blood spot samples (Job Aid). PATH: Seattle; 2005. Available from: <https://www.path.org/resources/rbp-eia-collecting-processing-and-handling-venous-capillary-and-blood-spot-samples-job-aid/> [74]
- A step-by-step video on preparing and processing DBS is available from: <http://www.jove.com/video/52619> [75]

Strengths of DBS testing

- Less invasive
- Requires minimal staff training, even non-medical trained staff can collect samples
- Self-sampling is possible
- Samples do not have to be centrifuged, separated, or frozen immediately
- Easy and cheap transport, filter cards can be sent via regular mail
- Storage of samples during field procedures does not require immediate freezing
- Multiple assays may be performed from a single drop of whole blood
- Serological and molecular testing possible, validation studies available
- Sensitivity/specificity almost as high as for venous blood.

Weaknesses of DBS testing

- Requires manual pre-processing steps in the lab: additional staff/time needed
- Existing commercial assays not yet validated for use with DBS specimens by the manufacturers, therefore results of the testing will not be returned to patients without additional testing (use of in vitro diagnostic off label)
- Storage conditions might have an impact on the accuracy of results
- The use of a DBS specimen may require adjustment of the assay cut-off to determine test positivity for serological screening, because DBS specimens use only a small volume of blood
- DBS samples have to dry prior to further transportation, which may be difficult when using mobile units
- DBS sample may not be adequate for multiple assays.

7.3 Storage and transport of specimens

7.3.1 Venous blood samples

Centralised testing of venous blood samples requires transportation of specimens to the laboratory for processing. This can be done using a specimen transportation system in the country (courier or similar services to take samples to the laboratory). Serum needs to be frozen or stored in a refrigerator if freezing facilities do not exist. Repeated freezing and thawing must be avoided.

Countries need to ensure the safe transport of potentially infectious specimens. To help freight contractors to comply with regulations, WHO has developed guidance and training resources on the transport of infectious substances. The *Guidance on regulations for the transport of infectious substances* [76] is updated by WHO on a regular basis, and most countries have issued national guidelines.

Diagnostic specimens collected in a prevalence survey for HCV and other blood-borne viruses fall into UN 3373 'Biological substance, Category B.':

Diagnostic specimens, assigned to UN 3373, are human or animal materials that are being transported only for the purpose of diagnosis or investigation. Such materials include excreta, blood and its components, as well as other tissues and fluids.

All UN 3373 substances must be transported in compliance with 49 CFR, Part 173.199 or *IATA packing instruction 650*. According to the *Transport packaging for UN 3373 Substances*, any packaging of infectious substances needs to consist of three layers.

Further details can be found here:

- EHES manual, part B – 6.1.1.6 Blood sampling processing [71]
- World Health Organization. Guidelines on hepatitis B and C testing. WHO: Geneva; 2017 (Summary diagnostic performance of DBS specimens for serological and NAT testing, p. 88, Table 13.1.) [77]
- WHO guidance on regulations for the transport of infectious substances [76]

- WHO website on transport of infectious substances, available from: <https://www.who.int/ihr/capacity-strengthening/infectious-substances/en/>

7.3.2 Dried blood spots

DBS need to be dried at room temperature for at least three to four hours before packaging and transport. The filter cards can be stored once the drying process is complete. If molecular testing is planned, deep freezing of the filter cards to a temperature of -20 °C or lower is required and should take place as soon as possible [77].

For storage, the filter paper card should be put in a single, gas-impermeable zipper bag containing several desiccant packs to protect the specimens from moisture. Optionally, a humidity indicator card can be added. Preferably, DBS specimens should be stored as soon as possible after transport to the laboratory. For serological testing, it is sufficient to store them at 4 °C for up to three months (if testing is planned soon); they should not be frozen unless prolonged storage is foreseen.

Processing and testing of DBS specimens should be conducted in an experienced and qualified centralised laboratory.

Frozen DBS specimens should be transported on dry ice. For filter cards initially kept at ambient temperature, a triple packaging system should be used, which consists of the zipper bags as the inner containers as well as an inner and an outer envelope. According to the last updated guidance by WHO, DBS are not subject to dangerous goods regulations [78]. No content markings are required on the outer envelope for shipment by regular mail but the international biohazard symbol must be affixed to the primary inner container [75].

Few studies have evaluated the impact of different storage and transport conditions on the accuracy of results from DBS specimens. Instability of results may appear when specimens were stored for a prolonged period of time (more than 14 days) at high temperatures (room temperature and above) and in humid conditions. A 2017 study found no connection between storage and serological test results [79], but HCV-RNA concentrations from DBS may slightly decrease when stored at room temperature [80]; the reported positivity of test results remained valid [81].

Further reading:

- World Health Organization. Guidelines on hepatitis B and C testing. Geneva: WHO; 2017. Summary diagnostic performance of DBS specimens for serological and NAT testing (Table 13.1, p. 88) [77]
- WHO guidance on regulations for the transport of infectious substances [76]
- WHO website on transport of infectious substances, available from: <https://www.who.int/ihr/capacity-strengthening/infectious-substances/en/>.

7.4 Long-term storage of collected samples

As a minimum requirement, all samples should be stored until the end of the study in order to allow for repeated testing if test results were inconclusive. If there is sufficient storage capacity, residual samples should be stored in a serum bank for future use. In national HCV surveys, extra samples could be collected and stored to allow for additional testing (e.g. HCV genotype, HBV/HIV coinfections) at later stage. If samples are collected and stored for later use, ethical and data protection approval is required to cover additional use; this needs to be included in the informed consent form.

Stored venous blood samples can be used for retrospective testing. It is important that the storage conditions are met: for long-term storage, serum and plasma samples must be stored at -20°C, preferably at -70 °C or below. There seems to be no problem with testing specimens stored in deep freezing for up to 10–15 years.

For DBS samples, storage at -20 °C or below is recommended, but there is limited evidence on the effect of long-time storage on testing accuracy [70, 77].

7.5 Types of testing laboratories

Testing laboratories, both centralised or decentralised, should be accredited and certified. The most important criterion for the selection of a laboratory should be its performance in external quality assessment programmes. A prerequisite for laboratories to become accredited is to have a documented quality management system.

Centralised testing in an accredited laboratory is recommended as the gold standard to ensure the comparability of test results. Testing in a central laboratory ensures that procedures are standardised and carried out in accordance with international standards. In addition, required tests (including PCR) can be performed at a high-quality level, with quality control mechanisms in place. Peer-review is usually available, and turnaround times are shorter since tests are run daily, which makes things also cheaper. In central laboratories, freezing and deep-freezing facilities are available, which simplifies the (long-term) storage of specimens.

Decentralised testing in several laboratories, e.g. in a laboratory near the study site, reduces packaging, transport and shipping, and may be cheaper than transporting the specimen to a central laboratory. Turnaround times from

sample collection to laboratory are reduced. If test results are to be given to participants, it might be easier to test in several decentralised labs and make use of the routine logistical arrangements.

A major disadvantage is that additional efforts have to be made to ensure the comparability and quality of test results. One way to ensure quality in decentralised testing is to send a small percentage of samples from each participating laboratory to a reference laboratory to cross-check the results before starting the survey [82].

If DBS specimens are collected, testing should be done in a centralised laboratory [83] with a proven track record in competently processing DBS specimens, including off-label testing.

If venous blood samples are collected, decentralised testing requires that quality control, qualified personnel and standard operating procedures are in place and adhered to in all participating laboratories. To ensure the comparability of results, similar testing methods have to be applied, the same types of assays have to be used, and high standards of quality have to be met.

Comment: Point-of-care testing with rapid tests is not recommended for population-based surveys of the general population due to the higher proportion of false-positive results that occur in low-prevalence settings. This would also result in a higher number of people who will have to undergo NAT testing in order to double-check the test results.

Table 6. Type of laboratory for testing the samples

	Minimum requirement	Gold standard
Venous blood samples	De-centralised accredited laboratories	Central accredited laboratory
Dried blood spots	Central accredited laboratory (preferred)	Central accredited laboratory

Further information is available here:

- EHES manual part A – 10.1 Selection of analytical laboratories
- EHES manual part B – 6.1.1.7 Maintenance of quality at the laboratory site
- World Health Organization. Laboratory quality management system: handbook. Geneva: WHO; 2011. Available from: https://www.who.int/ihr/publications/lqms_en.pdf [84]

8. Laboratory testing options

Nested survey collection

Residual sera testing

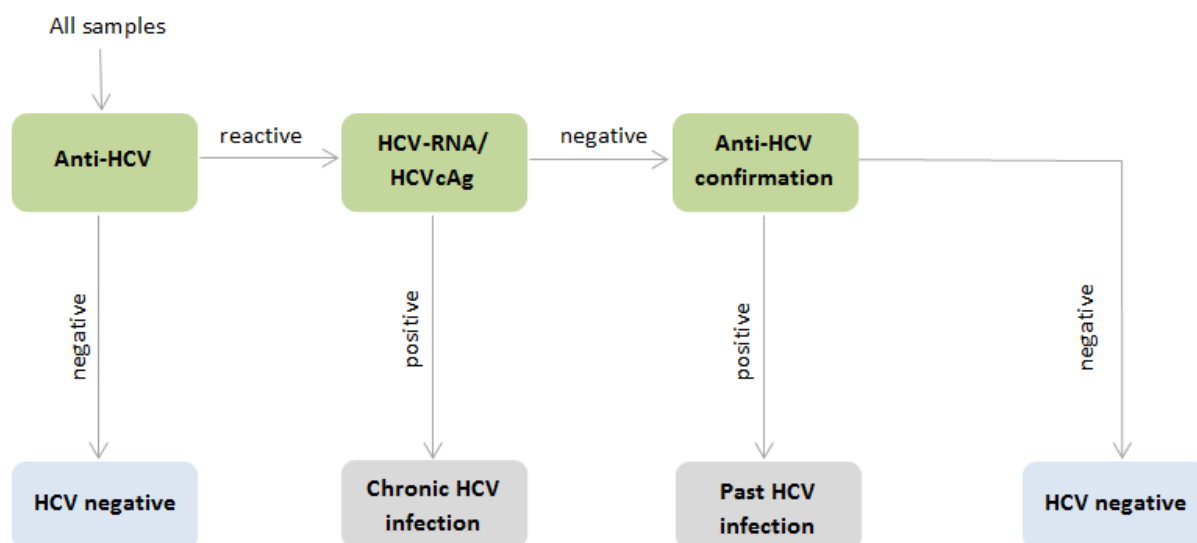
Stand-alone survey

To determine the combined prevalence of past and chronic infection, antibody testing as the first step of a testing algorithm would be sufficient. When testing in low-prevalence HCV settings, a higher proportion of false-positive test results has to be expected, due to the larger effect on the predictive value, as compared to testing in high-prevalence settings. To correct for false-positive anti-HCV screening tests, confirmation of the antibody by means of a second, more specific test (e.g. immunoblot) is recommended. False negatives may occur in the presence of HCV-HIV coinfection [86, 87].

To reach the objective of determining the prevalence of chronic infection in a population, active infections need to be identified. This should be done by sequential testing: after a reactive HCV antibody (anti-HCV) serological test (enzyme immune assays or chemi-luminescent assay), a test for HCV viraemia is recommended (see Table 7).

The recommended testing algorithm and interpretation of test results is illustrated in Figure 6.

Figure 6. Testing algorithm and interpretation of results



The use of quantitative or qualitative nucleic acid testing (NAT) for quantitative or qualitative detection of HCV-RNA is recommended as the gold standard and the preferred method to identify viraemic infection.

An assay that can detect HCV core (p22) antigen (HCVcAg) and provides similar clinical sensitivity as NAT is a potentially less costly alternative to NAT to diagnose viraemic infection because existing serological testing methods can be used. HCVcAg testing is less complex to perform than NAT, and was suggested as an alternative test to confirm viraemic infection in settings where an expensive and technical challenging NAT is not available [88-90]. HCVcAg assays can perform with high sensitivity (>90%) and specificity (>98%) compared with NAT [91, 92].

Further details on the diagnostic accuracy of different anti-HCV assays, HCVcAg assays and NAT assays can be found here:

- World Health Organization. Guidelines on hepatitis B and C testing. Geneva: WHO; 2017 [77]
- Tang W, Chen W, Amini A, Boeras D, Falconer J, Kelly H, et al. (2017). Diagnostic accuracy of tests to detect hepatitis C antibody: a meta-analysis and review of the literature. BMC Infect Dis. 2017 Nov 1;17(Suppl 1):695 [93]
- Lange B, Cohn J, Roberts T, Camp J, Chauffour J, Gummadi N, et al. Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): two systematic reviews and meta-analyses. BMC Infect Dis. 2017 Nov 1;17 (Suppl 1):700 [94]
- Freiman JM, Tran TM, Schumacher SG, White LF, Ongarello S, Cohn J, et al., Hepatitis C core antigen testing for diagnosis of hepatitis c virus infection: a systematic review and meta-analysis. Ann Intern Med. 2016 Sep 6;165(5):345-55 [92].

Table 7. Gold standard and minimum requirements for testing algorithms

	Minimum requirement	Gold standard
Testing algorithm, 1st step	Anti-HCV	Anti-HCV
Testing algorithm, 2nd step	HCV core antigen	HCV-RNA

8.1 Recommended assays

The most sensitive serological and molecular tests – in terms of analytical sensitivity and clinical sensitivity – should be used. It is important to ensure that the results are accurate so that results can be compared with those obtained in other countries. This is also important if the test result is reported back to the participant. Any assay used should meet the performance criteria of the regulatory authorities. A wide range of tests meeting these requirements is commercially available.

8.1.1 Anti-HCV assays

Enzyme immunoassay (EIA) and chemiluminescence (CIA) assays that meet these requirements are commercially available. The CIA assays have been reported to have slightly higher sensitivity than the EIAs [86]. Third-generation EIAs and CIAs are the dominant HCV screening tools, and the specificity of EIA assays is reported to be greater than 99% in high-risk populations.

8.1.2 Combination HCV-antigen-antibody assays

In addition to antibody-only tests, fourth-generation antigen-antibody EIAs (also known as combination assays) for the detection of HCV are now available. These assays are based on the simultaneous detection of HCVcAg or nucleocapsid protein of HCV and anti-HCV. Although neither of these assays is as sensitive as an HCV antigen-specific assay, these assays have demonstrated improved sensitivity over HCV antibody-only assays, especially in the window period when antibodies are undetectable [95, 96]. These assays offer a reasonable alternative for testing for viraemic infection when NAT cannot be used because of costs, affordability, feasibility, or logistical challenges [86, 95, 96].

8.1.3 Tests for viraemic infection

For NAT testing, a range of qualitative and quantitative RNA tests are available. Several laboratory-based HCVcAg tests are commercially available for stand-alone detection of HCVcAg; more tests are in the pipeline [88, 97].

Comment: A major disadvantage of using DBS specimens is that assay manufacturers have not yet validated their commercial assays with DBS specimens, which is required for regulatory approval. An increasing number of studies have validated the use of DBS specimens to test for anti-HCV, HCV-RNA and HCVcAg on existing laboratory platforms [98, 99]. The WHO guidelines on hepatitis B and C testing summarise four systematic reviews and meta-analyses that evaluated the diagnostic accuracy and the impact and duration of different storage conditions of DBS specimens compared to venous blood specimens for HCV serological testing and NAT [77].

Further reading:

- UNITAID, World Health Organization. Hepatitis C – Diagnostic technology landscape. Geneva: WHO; 2015. Available from: http://unitaid.eu/assets/UNITAID-HCV_Diagnostic_Landscape-1st_edition.pdf [86]
- World Health Organization. Guidelines on hepatitis B and C testing. Geneva: WHO; 2017 [77]
- Cooke GS, Andrieux-Meyer I, Applegate TL, Atun R, Burry JR, Cheinquer H, et al. Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. *Lancet Gastroenterol Hepatol.* 2019 Feb;4(2):135-184. [18]

9. Informing participants about test results

Nested survey collection

Residual sera testing

Stand-alone survey

When using one of the prospective approaches, for ethical reasons it is strongly recommended that participants are informed about their test results. The exact procedure should be in accordance with the national approach to the governance of such studies. To simplify matters, the study team may consider to inform participants only about positive test results, as these require further testing, diagnostics and referral to clinical care. Participants must be informed about the extent of the study's information policy when signing the informed consent form, for example that they will not get contacted if their test results were negative. In order to ensure that test results reach all infected participants, the results should be delivered over the phone or by personal contact.

All participants who are diagnosed with suspected HCV infection should be referred on for further assessment in line with local-care pathways.

If a retrospective serum testing approach is used (i.e. samples were initially collected for a different purpose), it may not always be possible to return the results to the participants, which raises ethical questions. If testing was done on unlinked anonymous samples, informing the participants is of course impossible.

Tips from SPHERE-C pilot projects

Providing test results and scheduling the appointments for doing so can be very time-consuming. It is important to plan for this in terms of time and resources. If all participants will be informed about their test results (and not only those who tested positive), outsourcing this task to an appointment scheduling service should be considered.

10. Additional data and questionnaires

- Nested survey collection
- Residual sera testing
- Stand-alone survey

A minimum set of sociodemographic data should be collected for each participant, regardless of study design and format. This includes basic socio-demographics, i.e. information on sex, age (in years) at the time of blood sample collection, and a postal or geographical code. These areas can be boroughs, counties, or administrative regions.

In a prospective study, further sociodemographic information should include information on, for example, country of birth (both of the participant and his/her parents), year of migration to the current country of residence, highest level of education completed, and current net income. This information can be used to build a socioeconomic status variable that may allow for an analysis of social determinants of infection status [22].

Additional data may be captured using a short questionnaire, either self-administered or administered as an interview by trained study staff. The questionnaire may be either paper-based or digital, provided on a tablet or through a link to an online questionnaire. If both paper-based and digital formats are used, it is important to keep in mind that if there are certain rules that apply to the analysis of data collected with digital questionnaires.

Table 8. Additional data to be collected

	Minimum requirement	Gold standard
Sociodemographic information	<ul style="list-style-type: none"> Sex Age Geographic area of residence 	<ul style="list-style-type: none"> Sex Age Country of birth of the participant and his/her parents, including year of migration to the current country of residence Geographic area of residence Net income (in categories/income brackets) Education/highest level of education completed Current employment status
HCV testing and status	-	<ul style="list-style-type: none"> Ever tested Time of last test (month, year) Result of the last test
Risk factors	-	<ul style="list-style-type: none"> History of intravenous (IV) drug use Haemodialysis Blood transfusions (before routine screening was introduced in your country) Other interventions (invasive diagnostics, surgery) Organ transplantation (before screening was introduced in your country) Sexual risk behaviour (e.g. sex with HCV+ partner or other at-risk groups, e.g. MSM or PWID) Unsterile tattooing/piercing Previous imprisonment Family history of HCV Perinatal transmission

If a low number of cases is expected in the sample, it may not be possible to perform a meaningful risk factor analysis due to the lack of power. Instead, the collection of data regarding risk factors could be limited to people who tested HCV-antibody positive. This would both minimise burden to the participants and reduce costs, especially if data collection was done at the same time when positive participants would also receive their test results. Another option is to ask about previous treatments (when received, successful or not). A template questionnaire can be found in Appendix 6. The questionnaire should be piloted upon translation into the local language in order to check the validity of the translation. The piloting of the questionnaire should be done with sufficient time to allow for editing and revising.

Comment: Before including questions on risk factors in the questionnaire, a quick estimate of the expected number of cases should show whether the total will be sufficient for a meaningful analysis. Alternatively, researchers could limit questions on risk factors to people who were HCV-antibody positive.

11. Data protection and ethical issues

Nested survey collection

Residual sera testing

Stand-alone survey

11.1 Data protection

First of all, the survey team needs to get approval from the data protection commission. Data protection includes a number of issues, for example access to survey data and safe storage of personal information (names, addresses). Access rights should only be given to the people who actually need access to do their work, for example to provide linkage to care. If a stand-alone survey is conducted, make sure to check that data protection rules allow the transfer of data such as the participants' names and contact information from the sampling frame to the study site to ensure implementation of all recruitment steps (invitation letters, phone calls and/or house visits). The data set used for analysis should be anonymised and only be made accessible to a small group of the survey team. Other data protection issues that need to be addressed usually involve the duration of data storage and options for linking survey data to other databases/registers.

Further reading on data protection:

- EHES. Manual part B – Fieldwork Procedures. Available from: http://www.julkari.fi/bitstream/handle/10024/131503/URN_ISBN_978-952-302-701-5.pdf?sequence=1&isAllowed=y

11.2 Ethical issues

Surveys need to comply with national rules/legislation. In some countries, obtaining ethical approval for conducting an HCV survey might be necessary under national legislation. Since this can be very time-consuming, the survey team should identify all requirements (e.g. ethical approval under national legislation) early on in the survey planning phase.

It is important that study participants are adequately informed about the survey procedures. This includes information about potential risks and how the survey uses and stores samples and data. It is advised that the informed consent form should include a general description on possible future data use. This information should be given in writing and by personal communication.

Providing participants with information about their own health status, e.g. giving back test results, is considered the gold standard. This can be an important factor in motivating study participants when doing prospective data collection [71]. On the other hand, this approach is resource-intensive and time-consuming, as well as linkage to appropriate care. (In a survey based on the retrospective testing of samples, this may not be possible.) A detailed data sharing and management plan should be developed prior to data collection to ensure that those who test positive are linked to appropriate care.

If retrospective testing was chosen, it must be clarified whether the consent form of the original study covers the use of their specimens for further purposes. It should also be noted that even if consent from the individual participants may not be necessary in this type of study, ethical approval may be required.

More information about data protection and ethical issues in surveys can be found in the following document:

- European Medicines Agency. Guideline for good clinical practice E6 (R2). London: EMA; 2016. <https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice>

Tips from SPHERE-C pilot projects

Approval processes should be started early in order to be able to respond to additional requests.

- In Italy, the ethics commission required that all participants should be contacted by phone to personally receive their test result (HCV-positive and HCV-negative results).
- In Bulgaria, names and addresses from the sampling frame could not be shared with the study site due to data protection issues; instead, invitation letters were sent directly from the local register (which had access to the sampling frame); it was not possible to make follow-up phone calls.

12. Data management, analysis and reporting

Nested survey collection
 Residual sera testing
 Stand-alone survey

12.1 Data management

In parallel to the development of data collection tools and the questionnaire, the survey team should develop programmes for data entry and data verification. The checking programme should flag up possible data errors. Since questionnaire data and laboratory data are usually collected in two distinct processes, two databases for data entry should be created. Manual double entry of data (data are entered twice and by two different people) is defined as the gold standard for transferring data to an electronic format; the minimum standard would be to double-enter a subset (random sample) of the questionnaires, assuming that data are entered carefully and with high quality. Through the comparison of the two entries, mistakes can be identified and corrected by looking at the paper questionnaires.

If separate databases were created, the two datasets (questionnaire data and laboratory data) should be merged after data cleansing and using unique identifiers that link questionnaire and laboratory data.

12.2 Data analysis

The data analysis method chosen has to match the survey's sampling design.

Weights should be assigned to account for design and non-response. It is advised to involve a statistician for this step. For example, if there are fewer men among the respondents compared to the background population, the bias will have to be corrected by weighting the data (see below in 12.2.3). The data analysis should include a description of the sample in terms of sociodemographic characteristics and factors defining HCV risk groups. If population-wide statistics on the distribution of these characteristics at the national level are available, they should be included in the weighting procedure. In this way, the distribution in the sample can be adjusted to the national level. For example, if the sample in age group 50–70 years includes 70% women but in the population this age group has only 55% women, the women in the sample have to be down-weighted for this age group. As the minimum, the weighting procedure should take into account age, sex and regional or urban–rural distribution. In the analysis, crude and weighted overall estimates of the HCV prevalence and the fraction undiagnosed should be calculated, including 95% confidence intervals (taking into account the design of the survey). This should be accompanied by stratified analyses in accordance with sociodemographic and other relevant characteristics.

A dummy table for the generated results can be found in Appendix 7.

Further reading:

- Lohr SL. Sampling: design and analysis. 2nd edition. Boston, MA: Brooks/Cole; 2010.
- Stata: Stata Survey data reference manual, release 13. Available from: <https://www.stata.com/manuals/svy.pdf>
- UCLA, IDRE. Applied survey data analysis in Stata 13. Available from: <https://stats.idre.ucla.edu/stata/seminars/applied-svy-stata13/>
- Lumely, T. Analysis of complex survey samples. The Comprehensive R Archive Network; 2019. Available from: <https://cran.r-project.org/web/packages/survey/survey.pdf>

12.2.1 Non-response analysis

As part of analysing and reporting survey data, i.e. test results and additional data on participants, it is important to describe non-participants in a nonresponse analysis. Depending on what data are available on the non-responders, e.g. data from the sample frame and/or data from a non-response questionnaire, the non-responders can be described in terms of basic sociodemographics, and possible reasons for non-participation and risk factors can be given.

12.2.2 Dealing with non-response

Non-response may introduce bias in the estimates of prevalence and the proportion undiagnosed if the non-response is associated with any of these outcomes (selection bias). It is known that persons who are healthy and well-off are more likely to participate in health surveys, which may lead to an underestimation of the true prevalence [100]. If the survey is performed as a stand-alone survey with a focus on HCV infection, people with a known infection may be less likely to participate in the study because they already know their health status. This could lead to an underestimation of the true prevalence and an overestimation of the fraction undiagnosed. It is therefore important that efforts are made to prevent the loss of these participants by clearly explaining the value of participation, regardless of health status. In addition, incentives could be used to get this group to participate. Finally, it is necessary to consider the behavioural mechanisms of non-response in health surveys.

12.2.3 Weighting

If there is substantial non-response rate and the non-response is considered to be 'missing at random', using post-stratification weights is a good solution when analysing and reporting data.

Post-stratification rates intended as corrections for non-response are based on variables known for everyone in the original sample, e.g. sex, age and geographical affiliation. For example, if there are fewer male participants than in the distribution of the background population, then these are weighted more in the analysis in order to compensate for the low participation among men.

Weighting is also used to correct for non-response. If, however, the non-response rate is very high, it may not be possible to come up with a precise HCV prevalence estimate based on the survey results. One way out of this dilemma would be to not state a point estimate but instead present ranges of HCV prevalence. It is crucial to report openly on the limitations of the survey results.

12.3 Data reporting

When reporting data, it is important to bear in mind that the survey approach described in this protocol will generate a prevalence estimate for the general population; this should not be interpreted as an estimate of the national prevalence.

It is important to disseminate the survey results to the general public, national HCV stakeholders, policymakers, and healthcare authorities. Results can then be used for strategic planning and decision-making. Information should also be passed on to civil society organisations (e.g. liver patient organisations) and non-governmental organisations that play a key role in the national response to viral hepatitis. A dissemination plan should be prepared at an early stage of the survey process. Using a written report a means of dissemination can reach most target audiences. Other options are presentations, scientific journal articles, conference posters/presentations and national stakeholder consultations.

Reporting data and survey results should take place in accordance with formulated objectives that correspond to WHO indicators C.1b and C.6 of the ten core indicators identified by WHO [101]. The frequency of reporting will depend on national plans/strategies and planning details (milestones, timeline).

Further reading:

- EHES: Manual part A – Chapter 14. Dissemination and publicity
- World Health Organization. Consolidated strategic information guidelines for viral hepatitis: planning and tracking progress towards elimination. Geneva: World Health Organization; 2018 [101]. Available from: <https://apps.who.int/iris/bitstream/handle/10665/310912/9789241515191-eng.pdf>

13. Quality assurance of the entire operations

Nested survey collection Residual sera testing Stand-alone survey

Monitoring to ensure high quality of all aspects of the survey is an essential part of survey management. Monitoring should start at a very early phase of the survey planning process and continue throughout the whole survey.

In the beginning of the process, the survey team should identify all potential risks that might influence the quality of the planned survey so that risk control can become part of a contingency plan. It is equally important to ensure that multiple stakeholders (researchers, clinicians, lab experts, statisticians, patient groups, policymakers, civil society, etc.) are involved in the survey design and planning at an early stage, both at the national and local levels. Before launching the survey, the survey protocol should be peer-reviewed by qualified experts.

13.1 Piloting the protocol

The fieldwork phase should be piloted early on in the process to ensure that modifications can be made before launching of the actual survey. Certain methodologic tools (e.g. questionnaires, incentives, recruitment strategy and blood sampling strategy) should be tested during the pilot phase. Additional time for piloting should be allowed and added to the survey timeline (Chapter 14). If a national survey is planned, pilot-testing could take place in a smaller community before rolling out the nationwide survey.

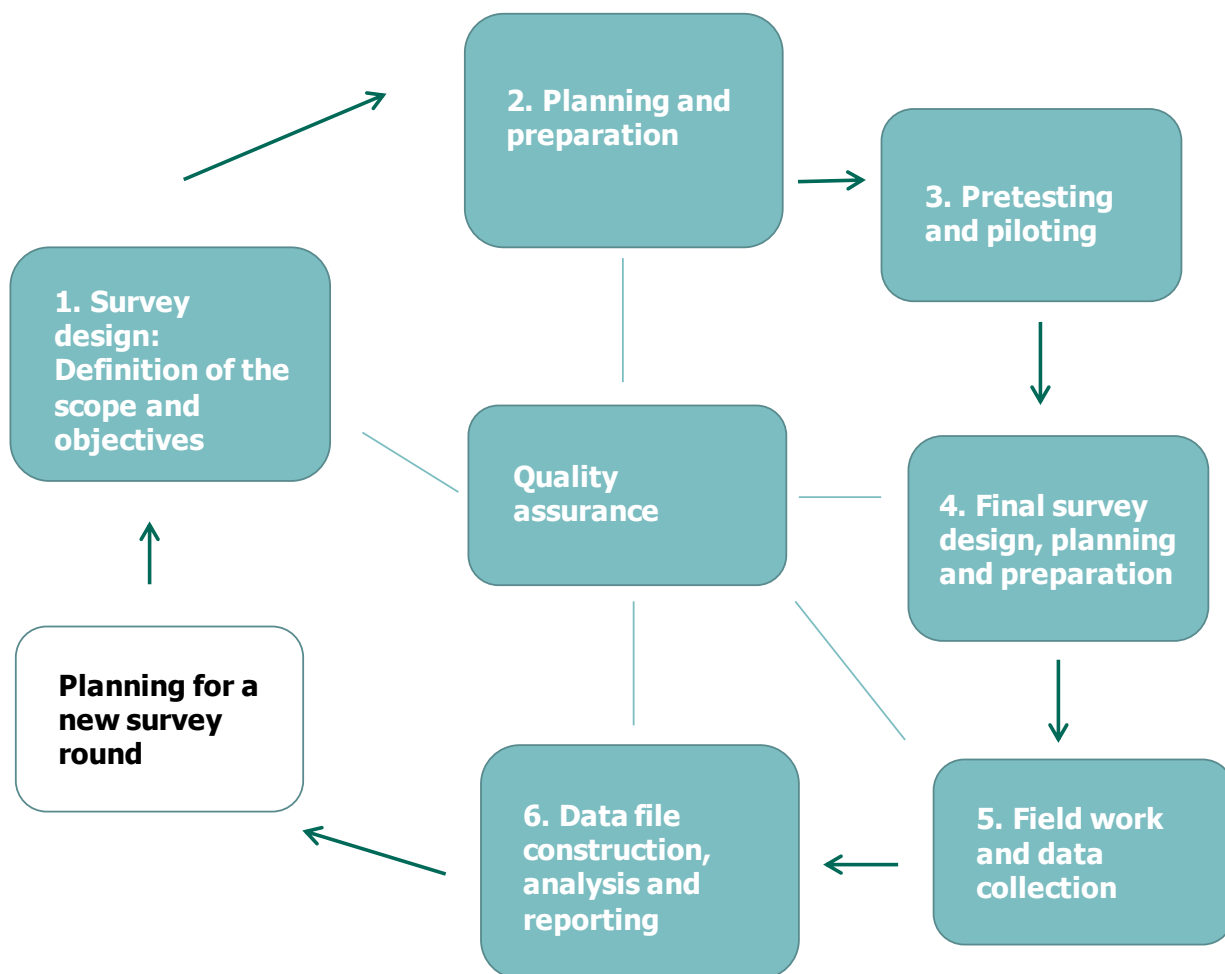
13.2 Quality assurance

Throughout the survey, quality assurance measures should be taken to detect and correct problems. Problems may arise with specimen collection, laboratory testing, training of survey staff, and data management.

If a prospective study is planned, basic components of quality assurance should include the following:

- Good survey management with a focus on quality and efficiency; this includes a well-defined survey management structure with clearly defined responsibilities of the survey staff and professional coordination that ensures that all objectives can be met, including agreed timelines and budget lines.
- Survey procedures that ensure standardised stable measurements.
- Survey personnel is familiar with, and trained on, standard procedures.
- The fieldwork phase was tested out during a pilot project that also covered data management, transportation of biological samples, and quality control.
- Quality control, which refers to measures taken to monitor the survey process, to detect problems at an early stage. The term 'quality control' also refers to action taken to correct detected problems.
- Evaluation of the achieved quality level.

Figure 7. Stages of the survey process



Source: EHES Manual part A – Planning and preparation of the survey [62]
 Note: Stages adapted from Franklin & Walker (2003), Czaja & Blair (2005)

14. Survey coordination, team composition and time management

- Nested survey collection
- Residual sera testing
- Stand-alone survey

14.1 Survey coordination

The survey coordinator should have project management skills to ensure the smooth running of the survey and collaboration between all stakeholders involved. If a nested survey is planned, it is important to ensure the collaboration between both teams, i.e. the one conducting the population-based survey (e.g. HES team) and the one running the hepatitis C prevalence survey.

Tips from pilot projects: retrospectively testing samples in Finland (*FinHealth2017* survey)

Retrospective testing is a fairly straightforward task that does not require a lot of coordination. During pilot-testing in Finland it became obvious quickly that a central coordinator who ensures collaboration between the HES team and the HCV prevalence survey team was essential for running the retrospective survey.

14.2 Team composition and training

Team composition depend on the chosen survey type. Possible positions to cover are:

- Leader
- Coordinator
- Healthcare personnel
- Lab technicians
- Receptionist
- Epidemiologist
- Statistician
- Data manager
- Recruitment expert

For the successful recruitment of participants and the collection of high-quality data highly trained and competent specialists are needed. Quality assurance relies on process evaluation of the survey segments; if re-training of staff is needed, processes should be in place that allow for additional training without endangering business continuity [102]. All staff involved in the survey should be well informed about the purpose of the survey; their tasks should be clearly assigned before data collection starts. Staff should also be provided with standard operating procedures (SOPs) describing the details of recruitment, data collection and data entry. If recruitment involves personal contacts, recruitment staff should have good social skills, be familiar with all aspects of the survey and trained in participation motivation. Staff working with data collection must be trained in obtaining informed consent (if required by national legislation) and know how to correctly collect and store the specimens. Data entry staff must be trained in not only how to correctly enter data from questionnaires into computers, but also know how to check for discrepancies, correct entry errors, and perform plausibility checks. Staff can be trained with role plays where the entire survey flow is practiced with the survey staff. This ensures that all SOPs and tasks are fully understood. Laboratory personnel must receive training on handling and testing of samples, as well as on correct sample storage and documentation of test results. Quality assurance of all laboratory procedures is of great importance to ensure valid results (Chapter 7.5); more details are available from:

- EHES manual part A – 10.1 Selection of analytical laboratories
- EHES manual part B – 6.1.1.7 Maintenance of quality at the laboratory site
- World Health Organization. Laboratory quality management system: handbook. Geneva: WHO; 2011. Available from: https://www.who.int/ihr/publications/lqms_en.pdf [84]

14.3 Time management

Regardless of the approach chosen, it is advisable to plan for additional time as the planning and execution of a prevalence survey may take longer than expected. This is particularly relevant if samples are tested retrospectively and one is confronted with unforeseen administrative tasks while carrying out a stand-alone survey. Prior to deciding on the different steps needed for a survey (e.g. recruitment, questionnaires), it can be helpful to engage with a pre-survey focus group to test different strategies. This should all take place during the planning phase and before deciding on the actual approach for the survey. All tasks to be carried out for the survey should be entered into a timeline (see Appendix 4). A budget calculator (see Chapter 15) can also be helpful to estimate the amount of time needed per participant.

15. Budgetary considerations

Nested survey collection

Residual sera testing

Stand-alone survey

There are several factors that influence the budget of an HCV prevalence survey. First of all, the choice of study design will directly influence expenses: a stand-alone survey will be more expensive than a nested survey or retrospective testing of samples already collected. A budget calculator (Excel) for the planning of a health examination survey (which could be used to nest a hepatitis C prevalence survey) is available at: http://www.ehes.info/rc/tools/time_cost.xls. (See also EHES manual, Part A, Section 16: Preparation of the survey budget. This budget calculator has been adapted for the SPHERE-C protocol to reflect items relevant for a hepatitis C prevalence survey.)

Ideally, when preparing the budget for a survey, the costs should cover the whole survey process:

- Planning and preparation
- Sampling
- Training of survey staff
- Piloting the survey
- Recruitment of participants
- Specimen/data collection
- Storage and transport
- Laboratory analysis
- Data entry and cleaning
- Quality assurance throughout the process
- Analysis
- Reporting/dissemination of the survey results

The actual unit costs for each of the items to be included in the budget will differ substantially across EU/EEA countries.

Other factors that will influence costs include:

- Geographical coverage
- Number and types of examination sites
- Duration of the data collection period
- Recruitment of participants:
 - Availability of sampling frames; will probably vary a lot.
 - Sample size
 - Materials for recruitment of participants; e.g. survey website, hotline, information material
 - Re-contact attempts and gathering information on non-responders
 - Incentives and/or reimbursement of travel costs; etc.
- Selection of survey staff:
 - National legislation in countries will vary in terms of who is allowed to collect a blood sample and/or to provide counselling on HCV
 - Statistical advice on national sampling frames; drawing a representative sample based on sampling frames
 - Survey management (including quality assurance and evaluation)
 - Additional costs for providing test results to participants (e.g. agreement with local/own medical doctor)
- Costs related to ensuring linkage to care for people found to be positive

The budgetary considerations need to include the above roles; they need to be adapted after careful consideration of the local/national settings.

Before calculating the budget, the amount of time required for fieldwork needs to be estimated (based on geographical situation, targeted sample size, number of clusters, etc.).

Further budget items to be considered are presented in Appendix 3.

16. Further reading

The below list contains key references for HCV prevalence surveys which are all in line with the recommendations in the SPHERE-C protocol.

- EHES: European Health Examination Survey. Website with a complete set of materials, manuals. Available from: www.ehes.info
 - EHES: Manual part A – Planning and preparation of the survey
 - EHES: Manual part B – Fieldwork procedures
 - EHES: Manual part C – European level collaboration
- SERISS: Synergies for Europe's Research Infrastructures in the Social Sciences. Available from: <http://seriss.eu/>
 - SERISS: Report on the use of sampling frames in European studies
 - SERISS: Report on sampling practices for the institutionalised population in social surveys
 - SERISS: Letter (and background) to request sampling for European surveys, available at: https://seriss.eu/wp-content/uploads/2018/10/SERISS-Deliverable-2.3b_Letter-to-request-access-for-sampling-for-European-survey-infrastructures.pdf
- Essential WHO publications on viral hepatitis prevalence surveys:
 - World Health Organization. Consolidated strategic information guidelines for viral hepatitis: planning and tracking progress towards elimination. Geneva: WHO; 2019. Available from: <https://www.who.int/hepatitis/publications/strategic-information-hepatitis/en/>. Includes web annex with a template protocol for surveys to estimate the prevalence of biomarkers of infection with the hepatitis viruses: tool for adaptation and use at country level. <https://apps.who.int/iris/bitstream/handle/10665/280099/WHO-CDS-HIV-19.3-eng.pdf>
 - World Health Organization. Guidelines on hepatitis B and C testing. Geneva: WHO; 2017.
 - World Health Organization. Updated guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. Geneva: WHO; 2016.
 - World Health Organization. Sample design and procedures for hepatitis B immunization surveys: A companion to the WHO cluster survey reference manual. Geneva: WHO; 2012.
- European Medicines Agency. Guideline for good clinical practice E6 (R2). London: EMA; 2016. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-6-r1-guideline-good-clinical-practice-step-5_en.pdf
- UNITAID, World Health Organization. Hepatitis C. Diagnostics technology landscape. Geneva: WHO; 2015. Available from: http://unitaid.org/images/marketdynamics/publications/UNITAID-HCV_Diagnostic_Landscape-1st_edition.pdf
- Recently conducted national HCV prevalence surveys:
 - Hagan, LM, Kasradze A, Salyer SJ, Gamkrelidze A, Alkhazashvili M, Chanturia G, et al. Hepatitis C prevalence and risk factors in Georgia, 2015: setting a baseline for elimination. BMC Public Health. 2019; 19: 480 [103]. Available from: <https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-019-6784-3>
 - Litzroth A, Suin V, Wyndham-Thomas C, Quoilin S, Muyldermans G, Vanwolleghe T, et al. Low hepatitis C prevalence in Belgium: implications for treatment reimbursement and scale up. BMC Public Health. 2019 Jan 8;19(1):39. [26]
 - Rosińska M, Parda N, Kołakowska A, Godzik P, Zakrzewska K, Madaliński K, et al. Factors associated with hepatitis C prevalence differ by the stage of liver fibrosis: A cross-sectional study in the general population in Poland, 2012–2016. [45]. Available from: <http://dx.plos.org/10.1371/journal.pone.0185055>
 - Bocquet V, Barré J, Couffignal S, d'Incau M, Delagardelle C, Michel G, et al. Study design and characteristics of the Luxembourg European Health Examination Survey (EHES-LUX). [60]. Available from: <https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-018-6087-0> [Example of a recent national health examination survey. Results are not HCV-specific but drawn from a general population survey.]
 - Verberk JDM, Vos RA, Mollema L, van Vliet J, van Weert JWM, de Melker HE, et al. Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis. 2019 May 28;19(1):470. [104]
- Recent examples of modelling studies on national HCV prevalence:
 - Harris RJ, Harris HE, Mandal S, Ramsay M, Vickerman P, Hickman M, et al. Monitoring the hepatitis C epidemic in England and evaluating intervention scale-up using routinely collected data. J Viral Hepat. 2019 May;26(5):541-551. [54]

- Koopsen J, van Steenberghe JE, Richardus JH, Prins M, Op de Coul ELM, Croes EA, et al. Chronic hepatitis B and C infections in the Netherlands: estimated prevalence in risk groups and the general population. *Epidemiol Infect.* 2019;147:e147. [51]
- Hofmeister MG, Rosenthal EM, Barker LK, Rosenberg ES, Barranco MA, Hall EW, et al. estimating prevalence of Hepatitis c virus infection in the United States, 2013–2016. *Hepatology (Baltimore, MD).* 2019;69(3):1020-31. [20]

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3. United Nations. Transforming our world: the 2030 Agenda for Sustainable Development. Resolution adopted by the General Assembly on 25 September 2015. New York: United Nations; 2015. Contract No.: A/RES/70/1.
4. World Health Organisation. Manual for the development and assessment of national viral hepatitis plans: a provisional document. Geneva: WHO; 2015.
5. World Health Organization. Global health sector strategy on viral hepatitis 2016–2021. Towards ending viral hepatitis. Geneva: WHO; 2016.
6. WHO Regional Office for Europe. Action plan for the health sector response to viral hepatitis in the WHO European Region [draft 2016]. Copenhagen: WHO Europe; 2016.
7. World Health Organization. Progress report on HIV, viral hepatitis and sexually transmitted infections, 2019. Accountability for the global health sector strategies, 2016–2021. Geneva: WHO; 2019.
8. European Centre for Disease Prevention and Control (ECDC). Hepatitis C surveillance in Europe – 2013. Stockholm: ECDC; 2015.
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- immunization programme in the Netherlands. Rijksinstituut voor Volksgezondheid en Milieu RIVM; 2010-03-09.
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Appendices

Appendix 1. Conducted and planned health examination surveys in EU/EEA countries

Country	National health surveys conducted between 2000-2017	Plans for 2018–2022
Austria	No surveys	Unknown
Belgium	2013–2015 collection of residual sera from 28 laboratories -> creation of serum bank for a multiple disease prevalence study [26]	2018 HES in connection to national HIS
Bulgaria	2015 National Risk Factor Survey, with few measurements (anthropometry and blood pressure)	–
Cyprus	No surveys	–
Czech Republic	2014–2015 Czech-EHES	2019 combining EHIS and EHES
Croatia	No surveys	–
Denmark	2007–2008 KRAM	–
Estonia	No surveys	–
Finland	2002, 2007 and 2012 FINRISK study 2000-2001 Health2000 2011-2012 Health2011 , follow-up survey for Health2000-survey FinHealth study in 2017	2019 <i>FinChildren</i> study (HES among children and adolescents) 2022 <i>FinHealth</i> study (HES among adults)
France	2006–2007 ENNS 2014–2016 ESTEBAN 2016 BaroTest (telephone survey with home-based self-collected DBS tested for HBV, HCV and HIV) [105]	–
Germany	2008–2011 DEGS , HES on adults. 2014–2016 KiGGS wave 2 (HES on children and adolescents and young adults)	HES on adults planned 2018-2020
Greece	2013–2014 HYDRIA 2013–2016 Hprolipsis hepatitis B/C and HIV survey (nested in EMENO HES on adults)[69] 2015-2017 survey on children and adolescents as extension of HYDRIA	
Hungary	No surveys	–
Iceland	No surveys	Unknown
Ireland	No surveys	–
Italy	2008–2012 OEC/HES	2018-2019 CUORE Project Health Examination Survey
Latvia	No surveys	Planned for 2016 including also blood pressure measurement but no blood sample collection
Lithuania	No surveys	–
Luxembourg	2016–2017 ORISCAV-LUX2 2013–2015 EHES-LUX 2007–2008 ORISCAV-LUX	–
Malta	2015-2016 SAHHTEK , main focus on diabetes but includes all EHES core measurements	–
Netherlands	2009–2010 NL de Maat 2006–2007 PIENTER 2-study 2016/2017 national population based sero-prevalence study [104]	–

Country	National health surveys conducted between 2000-2017	Plans for 2018–2022
Norway	No national HES. Several regional HESs have been conducted forming Cohort of Norway	–
Poland	2013–2014 WOBASZ II	Possibly, no fixed plans
Portugal	2012-2014 National HBV and HCV study [106] 2015-2016 INSEF	Planned for 2020
Romania	No surveys	–
Slovakia	2011 EHES survey	Planned for 2016, dependent on funding
Slovenia	No surveys	–
Spain	No national HES. 2008–2010 ENRICA study with focus on obesity but including all EHES core measurements	–
Sweden	No national HES	–
Turkey	HES 2015-2017 (household survey on non-communicable diseases)	
UK/England	Annually conducted Health Survey for England	Decision on continuation made until 2019

HES – Health Examination Survey; HIS: Health Interview Survey

Source: This table is based on the overview from the EHES website http://www.ehes.info/national/national_hes_status.htm (last updated 19 June 2018), but has been updated with a number of studies

Appendix 2. Population registers in the EU/EEA countries, as found by Poulain and Herm in 2013

Country	Type of register
Austria	Centralised register
Belgium	Centralised register
Bulgaria	Centralised register
Croatia	Information not available
Cyprus	Local civil registers
Czech Republic	Centralised register
Denmark	Centralised register
Estonia	Centralised register
Finland	Centralised register
France	–
Germany	Local registers, centralised register in some regions, centralised register of foreigners
Greece	Local civil register
Hungary	Centralised register
Iceland	Centralised register
Ireland	–
Italy	Local registers, centralised register in preparation
Latvia	Centralised register
Lithuania	Centralised register
Luxembourg	Centralised register
Malta	Local civic registers
Netherlands	Local registers linked online
Norway	Centralised register
Poland	Centralised register
Portugal	–
Romania	Centralised register

Country	Type of register
Slovakia	Centralised register
Slovenia	Centralised register
Spain	Centralised register
Sweden	Centralised register
Switzerland	Local registers, centralised register for foreigners
United Kingdom	–

For help getting access to registries for a survey, see memo from SERISS project, available at:

https://seriss.eu/wp-content/uploads/2018/10/SERISS-Deliverable-2.3b_Letter-to-request-access-for-sampling-for-European-survey-infrastructures.pdf

Appendix 3. Budget considerations for a nested or a stand-alone survey

Table 9. Budgetary considerations for surveys

Survey phase	Budgetary considerations to be included
Field work team	Healthcare personnel (e.g. nurses), lab technicians and receptionist (for registration of participants) if field study sites are used
Coordination	<ul style="list-style-type: none"> Coordinator Equipment (computers, software licenses, cell phones)
Planning	Epidemiologist, statistician, data manager, lab technician, PR person
Training	<ul style="list-style-type: none"> Health care personnel who does venepuncture/personnel doing the DBS specimen collection, lab technician trainer Lab materials
Piloting	All personnel and material posts. Material posts need to include the complete laboratory equipment needed during the pilot.
Sampling	Depends on strategy
Promotion	<ul style="list-style-type: none"> PR person PR materials
Recruitment	<ul style="list-style-type: none"> Person responsible for recruitment Incentives for participants Travel costs of participants to study site or of survey staff to households Phone calls to invite participants (tenderer responsible for the whole recruitment process)
Field work	<ul style="list-style-type: none"> Field work team, IT support Accommodation of the field team Laboratory equipment: <p>Venous blood sample collection:</p> <ul style="list-style-type: none"> Blood sampling vacuum tubes Needle for vacuum tubes Vacuum tube stands Needle disposal container Disinfection solution Swabs, gauze pads Skin tape Disposable gloves, latex and nitrile Tourniquets <p>DBS specimen collection:</p> <ul style="list-style-type: none"> Pre-punched filter cards Safety lancets Disposable gloves, latex and nitrile Disinfection solution Swabs, gauze pads Skin tape Desiccant packs

Survey phase	Budgetary considerations to be included
	<ul style="list-style-type: none"> • Humidity indicator cards • Gas-impermeable zipper bag • Equipment for package and shipping of the samples • Rent of examination sites • Transport of equipment to examination sites
Laboratory analysis and sample storage	<ul style="list-style-type: none"> • Lab technician • Tubes • Pipettes • Centrifuge • Costs of tests • For storage: freezer • Package materials and shipping of samples according to the specimen collected

Table 10. Indicative costs of types of laboratory HCV tests

Type of test	Test	Indicative costings	Reference
Serological test for the Detection of anti-HCV in human serum or plasma	EIA test	EUR 0.50-1.70	[77]
	EIA test, including additional laboratory infrastructure, equipment and quality control measures	EUR 7-8	[107]
Serological Hepatitis C core Antigen Test	Assay testing for HCVcAg	EUR 25-50	[77]
Qualitative nucleic acid test	Qualitative HCV NAT	EUR 40-47	[77]
Quantitative nucleic acid test	Quantitative HCV NAT	EUR 27-185	[77]
	qRT-PCR, including kit, staff, and laboratory extras	EUR 100-110	[91]

Appendix 4. Timeline (dummy example for a stand-alone HCV survey)

Task	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Develop HCV survey protocol												
Submit protocol for ethics review and data protection approval												
Pilot of HCV survey protocol												
Implement requested changes and finalise protocol (if ethics review takes >1 month potential for delay here)												
Draw sample from sample frame (if stand-alone survey)												
Conduct training of staff and plan for logistics												
Awareness-raising campaign												
Recruitment and management of participants (appointments)												
Data collection												
Data analysis												
Writing of report (publication if applicable)												

Appendix 5. Informed consent form

SPHERE-C: hepatitis C survey in Stara Zagora, Bulgaria

Information for study participants

Dear survey invitee,

The present form includes important information about the SPHERE-C Hepatitis C survey in Stara Zagora, how the survey is conducted and a description of what will be asked of you. Please read this information carefully. The steps will also be explained to you. If any parts of this form are not clear to you, please do not hesitate to ask the medical doctor who is collecting your informed consent and explaining the study. In order to participate, you will need to sign this form.

Content and objectives of the SPHERE-C Hepatitis C survey in Stara Zagora

You are invited to take part in the SPHERE-C Hepatitis C survey in Stara Zagora. This study is carried out to obtain information on hepatitis C in the general population in Stara Zagora by asking questions about health status and potential risk situations and measuring hepatitis C in the blood of each participant.

In Stara Zagora, there is to date very little information on the number of people living with hepatitis C in the general population, and this information will help future planning in the response in terms of prevention and control activities.

The survey is being conducted by the Regional Health Inspectorate, Stara Zagora in collaboration with the Municipality of Stara Zagora and epidemiologists from the Robert Koch Institute, the national public health institute in Germany, in the frame of a European project called SPHERE-C.

Selection of study participants

We aim at representing the adult population of Stara Zagora in the survey by drawing a random sample from the population aged 18 years and older. Your name and address were selected randomly from the local population register in Stara Zagora. Your participation is important to us, but please be assured that it is voluntary and you may leave the study at any time, and that your data will be kept confidential.

Measurements and questionnaire

During the survey, you will be asked to fill in a short questionnaire with questions regarding your sociodemographic background, your hepatitis C testing history and potential risk situations in the past. Further, a venous blood sample will be taken and tested for hepatitis C. The blood sample will be taken by qualified and specially trained nurses/doctors who are also trained to react competently to unforeseen situations in compliance with all the rules of the good medical practice.

To fill in the questionnaire and collect the blood sample, will take approximately 15 to 20 minutes of your time, during one visit.

Your test results

Your personal test results will be reported to you within the next two months. For this, you will get an appointment for a personal counselling on your test result by a doctor at the Regional Health Inspectorate in Stara Zagora. If the laboratory test was positive for hepatitis C, you will be referred for further clinical examinations and treated if necessary and possible.

Compensation

As a token of appreciation for your time spent on the study, you will receive a small gift from the study team.

Confidentiality and data protection

The data collected will be kept strictly confidential. They will be stored, analysed and handled in accordance with the European Union General Data Protection Regulation (GDPR) and the Bulgarian Law for Protection of Personal Data. No information that could be used to identify you will be provided to third parties. The results of this study will be published, but the publications will not include any information that could lead to your identification.

To ensure that the data collected from you remain confidential and that your data are protected, records and test results will be kept in a separate research file that does not include names or other information that could be used to identify you. As long as the data can be re-assigned to you have the right to ask what data was collected and saved from you, and you may correct it.

If you withdraw from the study, you may decide that your data and the samples should be eliminated. A withdrawal, or information and responses provided to the questionnaire, will not entail any legal consequences to

you. The Regional Health Inspectorate is responsible for safeguarding data protection in this study. If you are concerned about a possible violation of data protection or about any other issues regarding your data, you can contact the staff member, responsible for the data protection at the Regional Health Inspectorate of Stara Zagora, Mr. NN.

Storage of data and samples

The data and sample collected from you will be stored pseudonymously, this means separated from your person identifying information. Only the study coordinator will have access to the identification information. All data will be anonymised after completion of data collection and returning of test results. This means that your name and address will be removed and destroyed, latest by 1st March 2019. The blood sample collected from you will as well be destroyed after testing, latest 31st January 2019. Only the anonymised data will be used by researchers and will also be provided to researchers in the Robert Koch Institute collaborating on the survey.

Ethical approval

All aspects of this study have been approved by the Ethics Committee of the Regional Health Inspectorate of Stara Zagora.

Form for participants

Consent form

Name, first name ...

Participant ID ...

I have read and understood all of the information regarding this study and was orally informed about the study by ...

All of my questions have been adequately answered. I understand that if I have more questions or concerns about the survey or my participation, I may contact the person listed below.

I was informed that my participation in this study is voluntary and that I can withdraw my consent at any time during or after study participation. I may decide that my data and the samples should be eliminated. A withdrawal of consent does not need to be justified and has no negative implications. I was further informed that I have the right to ask what data was collected and saved from me, and that I have the right to correct it, as long as the data can be re-assigned to me.

I hereby give my consent to participate in the study. I agree to answer the questions in the questionnaire (or in an interview by trained study staff), and to give a blood sample for testing for hepatitis C.

I give my consent that the data provided may be processed, stored and used for the purpose of epidemiological analysis. I was informed that all data will be kept and stored strictly confidential and will be anonymised after the data collection phase of the study.

Stara Zagora

Signature participant

Confirmation by medical doctor

I hereby confirm to have adequately informed the participant about the contents and steps of the study including data protection issues in oral and written form.

Date/signature study doctor

Contact information for the SPHERE-C Hepatitis C prevalence survey

Principal investigator: Dr. N.N., MD; Director of the Regional Health Inspectorate

E-mail: nn@nn.org

Address: ... 6000 Stara Zagora, Bulgaria

Telephone: 042 604... or 042 602...

Data protection officer: Mr. N.N., Tel. 042 631 ...

Form for study documents

Consent form

Name, first name ...

Participant ID ...

I have read and understood all of the information regarding this study and was orally informed about the study by ...

All of my questions have been adequately answered. I understand that if I have more questions or concerns about the survey or my participation, I may contact the person listed below.

I was informed that my participation in this study is voluntary and that I can withdraw my consent at any time during or after study participation. I may decide that my data and the samples should be eliminated. A withdrawal of consent does not need to be justified and has no negative implications. I was further informed that I have the right to ask what data was collected and saved from me, and that I have the right to correct it, as long as the data can be re-assigned to me.

I hereby give my consent to participate in the study. I agree to answer the questions in the questionnaire (or in an interview by trained study staff), and to give a blood sample for testing for hepatitis C.

I give my consent that the data provided may be processed, stored and used for the purpose of epidemiological analysis. I was informed that all data will be kept and stored strictly confidential and will be anonymised after the data collection phase of the study.

Stara Zagora, ...

Signature participant

Confirmation by medical doctor

I hereby confirm to have adequately informed the participant about the contents and steps of the study including data protection issues in oral and written form.

Date/signature study doctor

Contact information for the SPHERE-C Hepatitis C prevalence survey

Principal investigator: Dr. N.N., MD; Director of the Regional Health Inspectorate

Data protection officer: Mr. N.N., Tel. 042 631 ...

Appendix 6. Template questionnaire

- A. Socio demographic information
1. Sex: Female () Male ()
 2. Age: _____
 3. Is your country of birth [country]? Yes () No ()
 - a. If no, please provide the following information:
 - i. *Your* country of birth: _____
 - ii. The year of migration to [country]: _____
 4. In which country was your biological mother born? _____
 5. In which country was your father mother born? _____
 6. Area of residence (e.g. postal code): _____
 7. What is your net income per month? [provide locally appropriate answer categories]
 8. What is the highest level of education that you have?
 - a. Elementary education (classes I-IV)()
 - b. Primary education (classes V-VIII) ()
 - c. Secondary education (classes IX-XII) ()
 - d. Higher education ()

(Bachelor – 4 years /Masters – 1-2 years)
 9. How many years of schooling do you have in total? _____ years
 10. What is your current professional status?
 - a. Student ()
 - b. Employed ()
 - c. Unemployed ()
 - d. Retired ()
- B. HCV testing history and knowledge of current HCV status
1. Have you ever been tested for HCV before? Yes () No () Do not know ()
 - a. If yes, when was your last test? (month and year) _____
 - b. If yes, what was the result of your last test?
 - i. Negative ()
 - ii. Positive ()
 - iii. Do not know ()

Additional questions, time, resources and data permitting

- C. Risk factors for HCV
1. Have you ever undergone surgery under general anaesthesia? Yes () No () Do not know ()
 2. Have you ever undergone haemodialysis? Yes () No () Do not know ()
 3. Have you ever undergone other invasive interventions? Yes () No () Do not know ()
 4. Have you ever undergone an organ transplant? Yes () No () Do not know ()
 5. Have you ever injected drugs? Yes () No () Do not know ()
 6. Have you undergone blood transfusion (prior to 1991) Yes () No () Do not know ()

7. Have you ever gotten a tattoo? Yes () No () Do not know ()
 8. Have you ever gotten a piercing? Yes () No () Do not know ()
 9. Is there anyone (among your close family or friends) who have been diagnosed with HCV? Yes () No () Do not know ()
 10. Have you ever been imprisoned? Yes () No () Do not know ()
 11. Have you had unsafe sex with an HCV positive sexual partner? Yes () No () Do not know ()
 12. Have you ever had sex with a man who has sex with other men or a person who has injected drugs? Yes () No () Do not know ()
- D. Treatment experience
1. Have you ever been treated for HCV? Yes () No () Do not know ()
 - a. If yes, was the treatment successful? Yes () No () Do not know ()

Appendix 7. Dummy tables for survey results

Table 10. Age-specific prevalence of chronic HCV by sex

Age	Chronically HCV infected (HCV-RNA+ & anti-HCV+)		Total	
	Male	Female	N	%
	N (%)	N (%)		
18-29	x	x	x	x
30-39	x	x	x	x
40-49	x	x	x	x
50-59	x	x	x	x
60-69	x	x	x	x
70-79	x	x	x	x
80-89	x	x	x	x
89+	x	x	x	x

Table 11. Undiagnosed fraction in the total study population

	Chronically HCV infected (HCV-RNA+ & anti-HCV+)	
	N	%
Ever tested for HCV before	x	x
Tested, but previous result was negative	x	x
Tested, and previous result was positive	x	x
Tested, but unaware of result	x	x
Unaware if tested	x	x
No response	x	x

Below you can find additional dummy tables for the HCV prevalence survey, time, resources and data permitting

Table 12. Distribution of risk factors of HCV infection in chronically HCV infected individuals

	Chronically HCV infected (HCV-RNA+ & anti-HCV+)		Entire study population	
	N	%	N	%
Other interventions (invasive diagnostics, surgery)	x	x	x	x
Haemodialysis	x	x	x	x
Organ transplantation	x	x	x	x
Blood transfusions before 1991	x	x	x	x
History of tattooing and/or piercing	x	x	x	x
Intravenous drug use	x	x	x	x
Close relations with an HCV-infected person	x	x	x	x
History of imprisonment	x	x	x	x

Table 13. Linkage to care and treatment of HCV positive individuals

	HCV positive (current or past)			
	Yes	No	Do not know	No response
Received treatment for HCV	x	x	x	x
Treatment successful	x	x	x	x

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