

Web Annex 2. Template protocol for surveys to estimate the prevalence of biomarkers of infection with the hepatitis viruses

In: Consolidated strategic information guidelines for viral hepatitis planning and tracking progress towards elimination

WHO/CDS/HIV/19.3

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TEMPLATE PROTOCOL FOR SURVEYS TO ESTIMATE THE PREVALENCE OF BIOMARKERS OF INFECTION WITH THE HEPATITIS VIRUSES

Tool for adaptation and use at country level

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ACRONYMS AND ABBREVIATIONS

CI	confidence interval
CLIA	chemoluminescence assay
DBS	dried blood spot
ECL	electrochemoluminescence assay
EIA	enzyme immunoassay
EQAS	external quality assessment schemes
GHSS	WHO Global Health Sector Strategy (on viral hepatitis)
HAV	hepatitis A virus
HBc	HBV core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HCW	health-care worker
HDV	hepatitis D virus
HEV	hepatitis E virus
Ig	immunoglobulin
M&E	monitoring and evaluation
MSM	men who have sex with men
NAT	nucleic acid test(ing)
PWID	people who inject drugs
QbyQ	question by question
RDS	respondent-driven sampling
RDT	rapid diagnostic test
SAE	serious adverse event
SOP	standard operating procedure
STEPS	WHO STEPwise approach to surveillance
SW	sex worker

QUESTIONS TO ADDRESS TO ADAPT THIS TEMPLATE INTO A NATIONAL PROTOCOL

To adapt this protocol to the needs of a specific country, national stakeholders need to answer eight key questions.

Question 1: Which hepatitis virus(es) require prevalence estimates? (See Objectives: A. Primary objectives)

- Hepatitis A virus (HAV) – estimating the prevalence of serological evidence of past or present infection
- Hepatitis E virus (HEV) – estimating the prevalence of serological evidence of past or present infection
- Hepatitis B virus (HBV) – estimating the prevalence of chronic infection
- HBV – estimating the prevalence of serological evidence of past or present infection
- Hepatitis D virus (HDV) – estimating the prevalence of current HDV/HBV coinfection
- HDV – estimating the prevalence of serological evidence of past or present HDV/HBV coinfection
- Hepatitis C virus (HCV) – estimating the prevalence of serological evidence of past or present infection and of chronic infection

Question 2: For which population(s) are (the) estimate(s) needed? (See Section V: Epidemiological methods: A. Population)

- General population
- Specific population (e.g. people who inject drugs [PWID], prisoners, health-care workers [HCWs], sex workers [SWs], pregnant women, men who have sex with men [MSM])

Question 3: What are the characteristics for which we are interested in examining the association with the presence of hepatitis biomarkers? (See Objectives: B. Secondary objectives)

- Geographical area (e.g. states, provinces, districts)
- Age groups for which age-specific estimates are needed
- Gender groups for which gender-specific estimates are needed

Question 4: Are synergies envisaged for the survey? (See Section V: Epidemiological methods: C. Synergies)

- Stand-alone hepatitis biomarker survey
- Survey conducted in conjunction with a survey that is conducted for other health issues (e.g. HIV, immunization)

Question 5: How will participants be sampled from the population? (See Section V: Epidemiological methods: E. Sampling procedure)

- Probability sample: simple random sampling
- Probability sample: systematic sampling
- Probability sample: cluster sampling
- Respondent-driven sampling (adapted to key populations)
- Convenience sample (to be avoided as it is not representative)

Question 6: What kind of techniques will be used for specimen collection? (See Section VI: In vitro diagnostic methods: A. Specimen collection)

- Venepuncture
- Capillary blood sampling
- Dried blood spots

Question 7: What kind of in vitro diagnostics will be used? (See Section VI: In vitro diagnostic methods: In vitro diagnosis)

- Rapid diagnostic tests
- Laboratory-based in vitro diagnostics

Question 8: What strategy will be used to return the results to survey participants? (See Section XII: Protection of human subjects: C. Benefits to individual participants)

- Immediate return of results with counselling and linkage to care
- Deferred return of results with counselling and linkage to care

PROTOCOL FOR A SURVEY TO ESTIMATE THE PREVALENCE OF BIOMARKERS OF INFECTION WITH THE HEPATITIS VIRUSES

Title

Protocol for a survey to estimate the prevalence of biomarkers of infection with the viral hepatitis viruses, including [HAV], [HBV], [HCV], [HDV], [HEV] *[delete what does not apply]*, among [add study population] in [add year] in [add geographical area]

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[Insert names, titles, affiliations and contact information]

In vitro diagnostics

[Insert names, titles, affiliations and contact information]

Implementing agency/ies

Sponsor

Source(s) of funding

Background

WHO estimates that in 2015, viral hepatitis led to 1.3 million deaths worldwide (1). The sequelae of chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) accounted for more than 95% of this death toll (1). In 2016, the World Health Assembly approved the first Global Health Sector Strategy (GHSS) on viral hepatitis (2). The GHSS on viral hepatitis calls for the elimination of viral hepatitis as a public health threat by 2030 (i.e. reducing the incidence by 90% and mortality by 65%, compared with the 2015 baseline). At country level, epidemiological assessments are needed to formulate an action plan for elimination. Since most chronic HBV and HCV infections are asymptomatic (3), biomarker surveys in the general population or in specific groups with a higher prevalence are necessary. In addition, biomarker surveys are needed for selected indicators of the WHO monitoring and evaluation (M&E) framework for viral hepatitis B and C, and they are the primary way by which the impact of vaccination is assessed (4). In addition, if surveillance information suggests that hepatitis A virus (HAV) endemicity may be intermediate with high reported rates of disease, the prevalence of antibodies to HAV by age group may help to guide decision-making on possible inclusion of hepatitis A vaccine in the routine immunization schedule (5).

In [country name], published studies and reports provide information on the epidemiology of viral hepatitis. [Insert summary relevant for HAV, HBV, HCV, HDV and HEV, as appropriate.] However, key information is missing, which would optimize the national plan. [Insert information needs on HAV, HBV, HCV, HDV and HEV.] Therefore, national stakeholders decided to conduct a biomarker survey that would include markers of infection with HAV, HBV, HCV, HDV and HEV. *[Delete what does not apply.]*

This protocol describes key proposed procedures to conduct a biomarker survey in [country name] in the field of viral hepatitis.

Objectives

Primary objectives

The primary objectives of the biomarker survey are as follows *[delete what does not apply]*:

Epidemiology of HAV infection

- Estimate the prevalence of serological evidence of past or present HAV infection (biomarker: total anti-HAV)* among [add study population] in [add year] in [add geographical area].

Epidemiology of HEV infection

- Estimate the prevalence of serological evidence of past or present HEV infection (biomarker: immunoglobulin (Ig)G anti-HEV or total anti-HEV) among [add study population] in [add year] in [add geographical area].

Epidemiology of HBV infection

- Estimate the prevalence of chronic HBV infection (biomarker: hepatitis B surface antigen [HBsAg]) among [add study population] in [add year] in [add geographical area].
- Estimate the prevalence of serological evidence of past or present HBV infection (biomarker: total antibodies against HBV core antigen [HBc], total anti-HBc) among [add study population] in [add year] in [add geographical area].

Epidemiology of HDV infection

- Estimate the prevalence of HDV/HBV coinfection (biomarker: HDV RNA in HBsAg-positive persons) among [add study population] in [add year] in [add geographical area].
- Estimate the prevalence of serological evidence of past or present HDV/HBV coinfection (biomarker: anti-HDV IgM in HBsAg-positive persons) among [add study population] in [add year] in [add geographical area].

* Or IgG anti-HAV (some assays measure only IgG)

Epidemiology of HCV infection

- Estimate the prevalence of serological evidence of past or present HCV infection (biomarker: antibodies against HCV [anti-HCV]) and the prevalence of chronic HCV infection (biomarker: HCV RNA or HCV core antigen) among [add study population] in [add year] in [add geographical area].

Secondary objectives

Key secondary objectives of the biomarker survey include the following *[delete what does not apply]*:

- estimating the proportion of persons with chronic HBV or HCV infection who are aware of their status;
- estimating the prevalence of hepatitis B e antigen (HBeAg) and/or HBV DNA in persons with chronic HBV infection* among [add study population] in [add year] in [add geographical area].

Quantifying the association between the presence of biomarkers and

- age, by age group [XX–XX years of age, XX–XX years of age, etc.]
- sex
- geographical areas (e.g. provinces, states)
- population subgroups (e.g. health-care workers [HCWs]).

Epidemiological methods

Population

[delete what does not apply]

- The study population will be the general population of the area considered from XX to XX years of age.

AND/OR

- The study population will be the specific population considered (e.g. persons who inject drugs [PWID] from XX to XX years of age)(6)

Design

- Cross-sectional survey

Synergies

[delete what does not apply]

- The survey will be a stand-alone hepatitis biomarker survey.

OR

- The hepatitis biomarker survey will be conducted along with another survey (e.g. HIV (7), tuberculosis, demographic and health surveys, WHO STEPwise approach to surveillance [STEPS], survey to estimate the prevalence of antibodies to measles or rubella virus infection). Thus, provision will be made to collect a sufficient quantity of blood specimens.

Operational definitions

[delete what does not apply]

This survey will use the following WHO-recommended case definitions (3):

* Mostly relevant for adults/women of childbearing age to guide programme decisions on the use of hepatitis B vaccine birth dose

HAV

- Serological evidence of past or present HAV infection: presence of total anti-HAV antibodies

HEV

- Serological evidence of past or present HEV infection: presence of IgG anti-HEV antibodies or total anti-HEV

HBV

- Serological evidence of past or present HBV infection: presence of anti-HBc antibodies
- Chronic HBV infection: presence of HBsAg* (3)

HDV

- HDV coinfection in persons with chronic HBV infection: presence of HDV RNA
- Serological evidence of past or present HDV coinfection in persons with chronic HBV infection: presence of anti-HDV IgM in HBsAg-positive persons

HCV

- Serological evidence of past or present HCV infection: presence of anti-HCV antibodies
- Chronic HCV infection: presence of HCV RNA (or HCV core antigen) (3).

Sampling procedure

Following guidance from WHO/UNAIDS guidance documents (6–10), the following sampling strategy will be used [*delete what does not apply*]:

Simple random sampling

- Investigators will sample individuals (one-stage sampling procedure) from the sampling frame using random number generators. The sampling frame will be sourced from [add an existing list of all individuals] (e.g. line list of individuals enrolled with a targeted intervention site of an HIV programme).

Systematic sampling

- Investigators will sample every [Nth] individual (one-stage sampling procedure) in the field. The sampling frame will be sourced from [add an existing list of all individuals] (e.g. sampling of pregnant women attending antenatal clinics).

Cluster sampling

- Using the total population of [country name] as a reference, investigators will list communities (e.g. census enumeration areas, neighbourhoods, villages), which are collectively exhaustive and mutually exclusive. These communities will be listed with their population size and the cumulated population size.
- From this list, investigators will select a number of communities in which clusters will be selected. The probability proportional to the estimated population size method will be applied to select communities (10).
- Within the selected communities, field workers will select clusters of a fixed number of households based on sample size calculation and expected household composition (from census data). To select households, they will use one of the following field methods of randomization [*delete what does not apply*]:
 - o Investigators will obtain (or establish) a list of all households from community leaders and select a fixed number of households at random (e.g. using a random number table).
 - o Investigators will obtain a map of the community using satellite mapping techniques, assign a number to each household and select a number of households at random.
- In each selected household, field workers will enumerate individuals residing in the household or those who slept in the household on the night prior to the visit. All eligible individuals will be invited to take part in the

* The WHO surveillance case definition for chronic infection is based on the absence of signs and symptoms of acute hepatitis and the presence of biomarkers of infection. (Coming across a recent infection in a cross-sectional population survey is unlikely.)

survey. A tally sheet will keep track of the selection process and document (a) offers to participate, (b) acceptances and (c) refusals.

- In case of non-respondent households, up to three visits on two different days will be organized. If after three visits the individual is unavailable, this person will be marked as a non-responder.

Respondent-driven sampling

- Investigators will recruit participants selected in the network of individuals already included in the sample (6,11,12). Such a sampling approach is recommended for hard-to-reach population groups, for which no sampling frame exists (e.g. PWID, MSM, SWs).

Convenience sampling

- Investigators will recruit participants from locations where they are easily accessible (e.g. health-care facilities).

Sample size calculation for the main estimate in the total sample (primary objective)

To address the primary objective of the survey, the sample size required for the main parameter (i.e. biomarker of interest) will be estimated on the basis of the following parameters:^{**}

- the expected prevalence of the biomarker in the surveyed population (proportion in percentage);
- the absolute precision (worst acceptable error, in percentage);
- the confidence interval (CI) needed (by default: 95% CI);
- the total population size (by default: this is assumed to be infinite. Corrections for finite populations apply only for very small populations, when the sampling proportion reaches around 10% of the total population);
- the design effect (1 for simple random/systematic sampling, 1.5 by default for cluster samples/respondent-driven sampling [RDS]);
- the expected response rate.

The sample size formula is:

$$n = [DEFF * N * p(1-p)] / [(N-1) * d^2 / Z_{1-\alpha/2}^2 + p(1-p)]; n_{total} = n + n * (1 - \%R)$$

DEFF = design effect. A conservative design effect of 1.5[‡] is used to account for an expected increase in variance due to clustering. Other studies using similar methodology have found similar design effects.

N = total population

$1-\alpha/2$ = desired confidence level of the estimate. A 95% confidence level corresponds to $Z_{1-\alpha/2} = 1.96$.

p = expected prevalence

d = absolute precision

R = expected response rate.

^{*} See more details in the 2015 WHO reference manual for vaccination coverage cluster surveys, annex B-1 for cluster surveys (10).

[†] See more details in the Introduction to HIV/AIDS and sexually transmitted infection surveillance, Module 4: Introduction to respondent-driven sampling (11).

[‡] Smaller design effects are sometimes possible because low prevalence may be associated with less clustering.

Table 1: Sample size table for various levels of expected prevalence and absolute precision, with 95% confidence intervals*

Expected prevalence	Absolute precision	Sample size	
		Design effect = 1	Design effect = 1.5
15%	5.0%	196	294
	3.0%	544	816
	1.0%	4 874	7 311
10%	4.0%	217	326
	2.0%	864	1 296
	1.0%	3 446	5 169
5%	3.0%	203	305
	2.0%	456	684
	1.0%	1 822	2 733
2%	1.0%	753	1 130
	0.5%	3 003	4 505
	0.2%	18 476	27 714
1%	0.5%	1 519	2 279
	0.2%	9 418	14 127
	0.1%	36 637	54 956

Table 1 outlines a number of sample size scenarios according to various levels of expected prevalence and desired precision.

For the age group of XX to XX years, a workable compromise will be to look for an expected prevalence of X%, with an absolute precision of X% and, when it applies, a design effect of X.X. **This will generate a sample size of XXX individuals (XXX after adjustment for XX% non-response).**

If a clustered sampling approach is used, a minimum of 30 clusters will be needed to reduce the design effect. Under that hypothesis, the number of individuals needed in each cluster will be XXX (=N/30).

Data collection

Each field team will consist of a supervisor, approximately X field surveyor(s) and X laboratory technician(s). In addition, the team will coordinate with logistics service providers for storage and transport of specimens.

Data collected

Information will be collected from the respondents (or their parents) on standardized [select: paper/electronic] questionnaires on which identifier stickers [if paper] will be placed to allow linking of paper questionnaires to blood samples. Information may be collected on [*delete what does not apply*]:

- demographic characteristics (e.g. age, gender, residence);
- history of vaccination for hepatitis A (for HAV biomarker survey only) and/or hepatitis B, including timely birth dose (for HBV biomarker surveys to evaluate the impact of hepatitis B immunization) preferably by card documentation or review of immunization records;
- knowledge of personal status in terms of HBV or HCV infection (e.g. past history of tests, test results, treatment history);
- being an HCW exposed to blood through patient care;
- having received a blood transfusion in the past.

See Appendix 3 for a template questionnaire.

* A desired absolute precision of 1% represents 6.7% relative precision for an expected prevalence of 15% (sample size: 4874), 10% relative precision for an expected prevalence of 10% (sample size: 3446), etc. The same absolute precision leads to less relative precision as the expected prevalence decreases, and the estimated sample size will decrease. To maintain good levels of relative precision as the expected prevalence decreases, the estimated sample size will increase.

For cluster surveys, see Appendix 4 for a template household log sheet to document the response rate and completed questionnaires and tests in each cluster.

Detailed information on all risk factors for acquisition of HBV and HCV infection will not be collected. Collection of such information is mostly relevant in the context of surveillance for acute hepatitis to understand current transmission pathways. Cases of acute hepatitis denote recent infections for which the likely time period when the subject was infected can be identified. In contrast, for persons with chronic infection or with serological evidence of past or present infection, the timing of the infection cannot be estimated. Hence, it is not possible to determine if the exposure occurred before infection leading to infection or if infection occurred before exposure, leading to exposure. As a result, studies comparing the prevalence of biomarkers in persons with and without specific exposures must be interpreted with caution to identify modes of transmission. They can even waste resources and generate misleading information. Finally, for the purposes of human subject protection, questions that are sensitive (e.g. illicit drug use or sexual practices) would not be adapted to community-based surveys as ensuring confidentiality during home visits under field conditions may be challenging.

Data collection procedure*

Trained field workers will (a) visit the place of participants' recruitment (e.g. home of the participants, health-care facility), (b) provide information about the survey and relevant documents, including pre-test counselling (see Appendix 5), (c) offer participation, (d) obtain informed consent, (e) collect the information through direct interviews with consenting individuals, and (f) collect blood specimens and, according to the strategy, (g) perform rapid testing on site *[delete what does not apply]*, (h) give results with post-test counselling information *[delete what does not apply]*.

Field workers will revisit households three times on two different days in case of absence of the selected individual. If the selected individual remains absent after three visits, it will be counted as absent (non-response).

Non-response/absentee forms will be used to collect demographic information (age, sex, geographical location and vaccination status, if possible) for individuals who refuse to take part or who cannot be included because of absence.

In vitro diagnostic methods

Specimen collection

[delete what does not apply]

Investigators will use the specimen collection techniques listed hereafter, in accordance with WHO guidelines on drawing blood (13). Field workers will make a maximum of two attempts to collect blood from participants.

Sampling of venous blood through venepuncture

Venepuncture is the preferred technique for blood collection. It allows collection of a volume of blood that permits testing with more than one assay in a laboratory.

The quantity of blood to be drawn will depend on the number of analytes tested (about 1 mL of serum for each test, e.g. a tube of 10 mL of blood to recover 5 mL of serum). The following tubes will be used *[delete what does not apply]*:

- Serum separator tube (if only serology is envisaged)
- EDTA tubes (only for nucleic acid testing [NAT], if NAT is envisaged).

Field workers trained to conduct venepuncture will collect specimens of blood from a vein (preferably the cubital vein or alternatively the dorsal vein of the hand) using the following general methods:

- Hand hygiene;

* In high-income countries, this may be adapted/modified for self-data collection (e.g. mailed questionnaire, online data collection).

- Use of disposable gloves;
- Disposable specimen collection devices for each participant;
- Preparation of the skin over the puncture site with an alcohol swab in a circular motion moving from the centre to the periphery and allowing the skin to dry. Care will be taken not to touch the area again once it has been prepared.
- Use of a tourniquet tied above the antecubital fossa, or forearm if the dorsal vein of the hand is used. Tourniquet time will not exceed 1 min as it causes haemoconcentration and can increase values of protein-based analytes.
- Puncture of the vein with the bevelled edge of the needle; the blood will flow immediately once the needle is in the vein. (If available, flashback needles are recommended to visualize the flow of blood in the needle hub.)
- Immediate release of the tourniquet after commencement of blood flow;
- Collection of blood until the required amount has been collected or until exhaustion of the vacuum;
- After completion of collection, withdrawal of the needle with the alcohol swab over the puncture site;
- Firm pressure applied over the puncture site until bleeding ceases;
- Use of an adhesive bandage;
- Disposal of sharps waste in a puncture- and leak-proof container.

The WHO job aid for best practices in venepuncture for laboratory testing and the WHO toolkit to prevent needle-stick injuries* will be used for training (14). In case of needle-stick injury, post-exposure management will be applied based on the national guideline,† which may include (a) testing of the source patient, and (b) offer of hepatitis B vaccination, and/or (c) post-exposure HIV prophylaxis, if indicated.

Sampling of capillary blood through finger-prick

Samples of capillary blood will be taken in the field for use with RDTs that have been validated for use with capillary blood.‡ (See Appendix 8 and WHO job aid (15).) In small children, however, heel-stick may be used to draw capillary blood into a small container for the collection of capillary whole blood. The following general methods should be used to collect blood:

- Hand hygiene;
- Use of sterile, disposable gloves;
- Use of disposable lancets for each participant;
- A fingertip (or heel for infants) will be rubbed and kneaded to help withdraw blood (the side of the participant's finger closest to the thumb will be rubbed).
- The fingertip (middle or ring finger) will be swiped or swabbed by using a sterile swab impregnated with alcohol. Time will be allowed for the alcohol to dry.
- The massaged place on the fingertip will be lanced with a single-use sterile lancing device.
- A hanging blood drop will be allowed to form without applying too much pressure. The first drop of blood will be wiped away.
- Drop(s) from the puncture will be collected into a small tube used for collection of capillary whole blood (according to the quantity needed).
- A cotton ball will be given to the participant to press on the puncture.
- The puncture site will be covered with an adhesive bandage.
- All waste will be disposed of in a puncture- and leak-proof container.
- Sometimes multiple punctures might be needed to obtain enough blood. A person will be allowed a maximum of three punctures with a lancet.

* Protecting healthcare workers: preventing needle-stick injuries toolkit. Geneva: WHO; 2005 (http://www.who.int/occupational_health/activities/pnitoolkit/en/, accessed 16 November 2018).

† WHO does not have guidelines for needle-stick management.

‡ Participants in whom the initial test results are reactive can then undergo a venepuncture for additional testing in the laboratory.

Dried blood spot (DBS) specimens

DBS can be used for the transport of specimens to the laboratory. However, the quantity of specimen collected will be limited. Cross-contamination must be prevented during collection of DBS specimens.

In the field, DBS specimens will be routinely prepared by pouring approximately 75 µL of capillary or venous blood (approximately one drop of blood) with a precision pipette onto the filter paper and allowing the spots to air dry. These will be packaged in individual bags to prevent-cross contamination. A desiccant will be used during transportation. (See Appendix 9.)

Use of DBS specimens implies the use of laboratory-based assays. In the context of “off-label” use of in vitro diagnostics (not authorized for returning medical results), the test results will not be returned to patients without additional testing.

Specimen processing, storage and transport

[delete what does not apply]

Sampling of venous blood through venepuncture

After each specimen is collected in the field, it will be placed in a test-tube stand and processed as follows:

- Specimens collected in a serum separator tube will be required to stand for a minimum of 60 min at room temperature to allow a clot to form prior to transportation under refrigeration to the nearest laboratory.
- Specimens collected in EDTA will be taken to the nearest laboratory under refrigeration (2–8 °C) where they will be centrifuged.

Specimens will be transported in batches to the laboratory for further processing once a day. At the peripheral laboratory, aliquots of serum will be put into small vials. All specimens will then be stored at 2–8 °C if testing is performed within 5 days, and at –20 to –70 °C if testing is to be performed later. Labelling will be done with identifier stickers that will allow links with the paper questionnaires and prevent errors (e.g. use of bar codes). Freezing/thawing cycles will be avoided. The temperature will be monitored.

- The phlebotomists will coordinate with the logistics service provider to ensure that the shipment is picked up at the right time and transported under defined conditions.
- Logistics services will be responsible for supervising the cataloguing and storage of the specimens at the correct temperatures during the transportation process.
- The laboratory project management team will train the logistics services.
- Laboratory staff will be responsible for supervising the storage of the specimens once they reach the laboratory where testing will be conducted. The number of boxes, condition of the vials, and time of receipt of specimens in the laboratory will be noted upon receipt of shipments at the laboratory. Staff will use a checklist as follows to document:
 - ✓ time the shipment was delivered
 - ✓ number of boxes in the shipment
 - ✓ number of specimens in each box
 - ✓ freezing of specimens, and temperature at which freezing occurred
 - ✓ damaged or broken specimens.

Quality assurance and quality control (internal) of specimen processing will proceed according to established policies and standard operating procedures (SOPs) of the laboratory. Care will be taken during training to encourage open and accurate reporting and documentation of cold chain interruptions.

Sampling of capillary blood through finger-prick

- Usually, capillary blood sampling is for immediate use with RDTs. Capillary whole blood may either be transferred directly from the finger-stick to the test device using the specimen transfer device provided by the manufacturer, or may be transferred (if not used immediately) into a micro-vial specifically designed to collect capillary whole blood.

DBS specimens

- DBS specimens will be processed as per the instructions of the manufacturers or of the laboratory in case of off-label use.

In vitro diagnosis

To ensure that test results can be returned to participants, testing strategies (i.e. the sequence in which the various assays will be used) will follow the WHO testing guidelines for viral hepatitis (16).

Testing strategies for HBV

Chronic HBV infection

The testing strategy for the diagnosis of chronic HBV infection in adults and children >12 months of age will consist of a test for HBsAg (either RDT or laboratory-based immunoassay), see Appendix 10: Testing strategy for the diagnosis of HBV infection in biomarker surveys. Participants who are HBsAg reactive will be reported as positive (compatible with HBV infection). Participants who are HBsAg non-reactive will be reported as negative.

- In settings or populations with an HBsAg seroprevalence $\geq 0.4\%$, WHO recommends a single serological assay for the detection of HBsAg.
- In settings or populations with an HBsAg seroprevalence $< 0.4\%$, confirmation of HBsAg positivity on the same immunoassay with a neutralization step (for laboratory-based assays) or a second different RDT assay for detection of HBsAg may be considered.

Serological evidence of past or present HBV infection

If the objectives of the survey include estimation of the prevalence of serological evidence of past or present infection, the testing strategy may be modified to include (a) a first test for anti-HBc followed, if positive, by (b) a second test for HBsAg.*

Assay format

Box 1: WHO recommendations on the use of rapid diagnostic tests for the diagnosis of HBV infection (16)

- In settings where existing laboratory testing is already available and accessible, WHO recommends laboratory-based immunoassays[†] as the preferred assay format.
- In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended to improve access.

Testing strategies for HCV

HCV infection

Testing strategies for the diagnosis of HCV infection will consist of a first test for anti-HCV (either RDT or laboratory-based immunoassay), see Appendix 11: Testing strategy for the diagnosis of HCV infection. Participants who are anti-HCV reactive will be reported as positive (compatible with past or present HCV infection). Participants who are anti-HCV non-reactive will be reported as negative (no evidence of HCV infection).

* Unlike for HCV, it is not necessary to identify patients with serological evidence of past or present HBV infection to identify those with current HBV infection.

[†] Laboratory-based immunoassays include enzyme immunoassay (EIA), chemoluminescence immunoassay (CLIA) and electrochemoluminescence assay (ECL).

Participants who are anti-HCV reactive will proceed with supplementary testing for HCV RNA (qualitative or quantitative) or HCV core antigen. Participants who are HCV RNA or HCV core antigen positive will be reported as detected (compatible with current HCV infection). Participants who are HCV RNA or HCV core antigen negative will be reported as not detected (no current HCV infection).*

Assay format

Box 2: WHO recommendations on the use of rapid diagnostic tests for the diagnosis of HCV infection (16)

- In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, WHO recommends RDTs, followed by HCV RNA NAT or core antigen testing on reactive specimens.

Testing strategy for HAV and HEV

There is no specific testing strategy for HAV and HEV since only one type of biomarker is collected (total antibodies to HAV and/or total antibodies [or IgG antibodies] to HEV).

Testing strategy for HDV

Currently, WHO does not have a standard testing strategy for HDV. Such a strategy would need to be defined by the implementing team on an ad-hoc basis.

Testing algorithms

Following identification of the testing strategies, specific names of kits will be selected for each of the tests included in the strategy. Tests should meet minimum safety, quality and performance standards: assays should meet minimum acceptance criteria of either WHO prequalification of in vitro diagnostics or a stringent regulatory review for in vitro diagnostics. All in vitro diagnostics will be used in accordance with manufacturers' instructions for use.

The following assays will be used for the following biomarkers:

[List assays selected for each analyte, preferably WHO prequalified.]

Management of leftover specimens

Unless the research team has established a governance structure for leftover specimens, they will not be stored indefinitely and destroyed after viral hepatitis testing.†

Analysis plan‡

Data entry§

[delete what does not apply]

- Data entry will be done prospectively if electronic devices are used to collect data in the field.
- Epidemiological information collected on paper questionnaires will be entered in the computer using data entry software that will control for range and consistency. Identifiers will not be entered in the computer and unique codes will be used instead.
- Tests results will be entered in the computer using data entry software that will control for range (e.g. aberrant values) and cross-consistency of variables. Identifiers will not be entered in the computer and unique codes will be used instead.

Data cleaning

- Data managers will clean the epidemiological data file, including identification of duplicates.

* Unlike for HBV, it is necessary to identify patients with serological evidence of past or present HCV infection to identify those with current HCV infection.

† Investigators who would want to organize biobanks with leftover specimens should refer to the CIOMS guidelines

(<https://cioms.ch/shop/product/international-ethical-guidelines-for-health-related-research-involving-humans>) to set up an appropriate governance structure.

‡ This section assumes statistical sampling. To be adjusted in the case of convenience sampling.

§ See more details on digital data capture in the 2015 WHO reference manual for vaccination coverage cluster surveys (Annex I).

- Data managers will merge the epidemiological data file with the in vitro diagnosis data file using the unique identifier codes.
- Summary and frequency tables as well as visual representations of appropriate variables will be used to find impossible, implausible or missing values within the dataset.

Descriptive epidemiology

- Data analysts will calculate the crude prevalence using the number of participants as denominators and taking into account sampling weights as needed:
 - o for the overall population;
 - o for each population subgroup.
- Data analysts will calculate the 95% CIs taking into account the design effect.

The results of the analysis will be presented as per dummy tables. See Appendix 6: Dummy table shell for the analysis of a viral hepatitis biomarker survey.

Analytical epidemiology

If the protocol includes a step in terms of analytical epidemiology *[delete what does not apply]*:

- Data analysts will compare the prevalence of biomarkers in populations with and without the selected characteristics through the calculation of prevalence ratios and their 95% CIs, taking into account sampling design effect and weights as needed.*
- The results of the analysis will be presented according to the framework proposed by the set of dummy tables. Appendix 7: Dummy table shells to compare groups with different characteristics in terms of prevalence of viral hepatitis biomarkers will be used for the comparison of different groups in terms of prevalence of viral hepatitis biomarkers.
- If data were collected to examine factors associated with vaccination (e.g. home birth versus health facility birth), analytical epidemiology could examine missed opportunities for vaccination.† *[Delete what does not apply.]*

Quality assurance

Field work

Quality assurance procedures will include:

- translation and back-translation of questionnaires and tools (as per ethics committee requirements);
- pilot-testing of the questionnaire and tools;
- preparation of a “question by question” (QbyQ) guide for field workers and SOPs;
- training of field workers in all procedures, including identifying the selected areas and households, collecting information, revisiting households three times on two different days in case of absence of the selected individual;
- standardization of the data collection procedures among different teams of field workers;
- spot-checking by supervisors to ensure adherence to SOPs;
- daily cross-checking of a number of questionnaires and a number of specimens (making sure the unique IDs match).

In vitro diagnosis

Quality assurance for in vitro testing will include record-keeping and documentation through SOPs, and process control (environmental monitoring, use of quality control specimens and external quality assessment schemes

* See instructions to calculate survey weights in the 2015 WHO reference manual for vaccination coverage cluster surveys (Annex J) (10).

† See more details on the analyses of missed opportunities for vaccination in the 2015 WHO reference manual for vaccination coverage cluster surveys (section 6.4.1) (10).

[EQAS])(17). (See Appendix 14: Tool for rapid assessment of laboratory capacity before a viral hepatitis biomarker survey)

The investigators will verify new in vitro diagnostics that have not been used earlier in the study laboratory through a verification study. See Annex 2 of the WHO guidance for procurement of diagnostics http://www.who.int/diagnostics_laboratory/publications/procurement/en/.

The tests conducted for the survey will be run together with a number of quality control specimens for which the results are known. These quality control specimens will be supplied by a reference laboratory. In countries with suboptimal laboratory capacity, 10–20% of specimens will be sent to a reference laboratory for quality control.

Data management and analysis

Quality assurance procedures will include:

- daily checking of missing/inconsistent values on paper questionnaires;
- double data entry;
- ensuring clear data management procedures (including good documentation of data cleaning and analysis) and proper training of data entry staff;
- prospective data cleaning and timely retrieval of missing data spotted by this process;
- making the anonymised database, codebook and analysis scripts available for review;
- peer review of the results of data analysis.

Limitations

Selection bias

Selection bias is possible, since some eligible participants will be more difficult to reach than others. Therefore, to minimize bias, efforts will be taken to return to households during the evening or weekend in case the randomly selected members are not initially available. Field teams will return to households up to three times on at least two different days in an attempt to reach the selected household member. The proportion of subjects approached for participation that agreed to take part will be computed and discussed. Moreover, available information (e.g. age, sex, vaccination status) will be compared between respondents and non-respondents. Previous surveys have been successful in minimizing selection bias by explaining the importance of the biomarker survey to community leaders and using their standing in the community to build awareness of the survey before it is conducted.

Information bias

Recall bias

Recall bias is possible when requiring participants to recollect medical, family, social and risk factors from the past. For example, the measure of association between hepatitis B biomarkers and vaccination status may be biased if the vaccination status is not based on vaccination cards or registries (recall biases). In addition, social desirability bias may occur due to potential stigmatization of PWID, MSM or SWs, leading to underreporting of injection drug use and same-sex sexual partners. However, sensitive questions will not be used in this survey out of consideration for protection of human subjects.

Interviewer bias

Interviewer bias is also possible if the knowledge of the study objectives, disease status or exposure status (if sensitive data are to be collected) influences data recording (observer expectation bias). The means by which interviewers can introduce error into a questionnaire include helping the participants in different ways (even with gestures), or putting emphasis on different questions. During interviewer training, emphasis will be placed on the importance of asking questions in a non-leading manner, and demonstrating and piloting useful ways of assisting participants to accurately recollect events. During training, staff will be instructed on consistent ways of asking

questions to minimize variations in survey administration between interviewers, and in recollection of information between participants. A standard script will be available for survey administration.

Sample size

Sample size calculation is based on hypotheses in terms of prevalence and, when it applies, design effect. Lower measured prevalence and/or larger design effect will lead to estimates that are less precise than anticipated. Furthermore, if not powered for these objectives, stratified analyses will lead to estimates with large CIs that will require careful interpretation. However, results from these analyses may trigger targeted surveys to identify specific exposure pathways.

Safety and monitoring

Anticipated adverse events may be related to collection of blood specimens. These include discomfort, bleeding, swelling, haematoma or infection at the site and fainting during or following collection. The participant should be seated and provided with sugary water. The participant will be provided contact information of the survey staff in the event of any concerns or adverse events following the blood draw. The nurse taking the blood specimen will be equipped with a basic first-aid kit to ensure optimal safety oversight. Since this is a non-interventional biomarker survey, no reporting of individual cases to regulatory agencies is planned. There are no serious adverse events anticipated and thus reporting will not be necessary. If a serious adverse event occurs, information regarding the nature of the event and the time that it happened will be reported to the principal investigator within 24 hours.

Linkage to care

Table 2: Framework for linkage to care of patients diagnosed with HBV and HCV infection during the survey (for tests not done on-site) (parts coloured in blue will not be the responsibility of the investigators but should exist in the setting where the study is conducted)

Stages of linkage and care	Levels of the health-care system	
	Primary health care (PHC)	Referral (district or national level)
Pre-test counselling	Counselling prior to specimen collection ↓	
Testing*	Collection of specimen →	Specimen testing ↓
Transmission of the results of the analysis of the initial specimen	↓	Report nominative results to the nearest PHC ←
Notification of the participant	Search for patient and notify of test results ↓	
Counselling	Counselling and referral →	↓
Additional testing and staging		Assessment of the patient ↓
Treatment		Initiation of treatment when applicable ↓
Monitoring	Continuation of follow up ↓	Follow up of patients as soon as possible ←
End-of-treatment evaluation (HCV)	↓	End-of-treatment assessment and assessment of viral response (HCV)

* In case of rapid diagnostic tests, testing is done at the primary health care level and patients immediately notified.

On the basis of the expected prevalence, it is expected that about XX patients would be identified with HBV infection while about XX would be identified with HCV infection. Patients will be linked to care and possible treatment according to a general framework (Table 2) and WHO guidelines for the treatment of persons with chronic hepatitis B or hepatitis C infection (18,19). For each step in the process, SOPs will be prepared through a consensus with experts, including clinicians and counselling specialists. Most children testing positive will not be eligible for treatment. Nonetheless, parents will be informed of the child's status and, when children test positive, counselled on how to prevent future transmission (e.g. vaccination of family members/future children, not sharing things that might have blood on it). An appropriate medical follow up will be offered for children who test positive.

Overall, the linkage to care during this survey may function as a pilot of the future programme for testing and treatment in the country (if a testing and treatment programme is not yet operational). The study team has the responsibility of ensuring that persons diagnosed are referred to care and treatment, and that such a system does exist. However, the investigators do not have to cover these costs themselves. The budget will be used to anticipate the cost in order to ensure that some entity will take responsibility for these costs (e.g. health system).

Protection of human subjects

This section describes the procedures in place to protect human subjects during the survey. This will include procedures at the community level and at the individual level. Procedures at the individual level will focus on three aspects, including (a) minimizing risks and/or inconvenience to participants, (b) maximizing benefits to the participants, and (c) securing informed consent from participants.

Community engagement and follow up

Planning the survey

The communities selected in the sample will be informed of the upcoming survey by the local health officers. The population will be informed through engaging their community leaders and using their standing in the community to build awareness of the survey before it is conducted. Additionally, there may be engagement of the press to ensure that the public knows about the survey and to provide key messages on hepatitis.

Community sensitization will use the elements described in the WHO factsheets on hepatitis B and C to avoid stigmatization of people who test positive (20,21), especially in communities where the prevalence is low and infected individuals could be singled out. These campaigns will particularly aim at illustrating that viral hepatitis is not necessarily associated with high-risk behaviour and that there is no risk of person-to-person transmission through casual contacts.

The random nature of selection of communities and individuals will be explained as part of this information so as to clarify any perception of deterministic choice that could lead to stigmatization (e.g. perception that a village was selected because it was problematic). In case a cluster survey is used, the team will explain to the population that the selection will be based on households rather than individuals and that, within each selected household, all individuals will be invited to participate. Care will be taken to make sure that the explanation of selection by chance is told in a way that can be understood in the specific cultural and educational context.

Feedback of the results

A full report will be shared with decision-makers; shorter reports will be sent to participating primary care facilities and a scientific article submitted to a peer-reviewed journal. Further use of the data is detailed in Section XIII: Expected benefits of the survey. The results of the survey will not be shared at the cluster level. It would have no meaning from a statistical point of view and could theoretically increase the risk of stigmatization. Furthermore, given the low prevalence of hepatitis, the number of individuals testing positive in each cluster is likely to be limited. This will facilitate follow up of persons testing positive in a confidential manner, which will also prevent the risk of stigmatization.

Risk/inconvenience to individual participants

- Field workers will be appropriately trained to minimize the negative consequences of blood collection. They will use sterile, single-use specimen collection equipment and follow WHO best practices for blood collection.
- Participants may undergo discomfort associated with blood specimen collection.
- The questionnaire will be administered privately in the house of the participant.
- Confidentiality will be protected through the use of unique identifier codes in the questionnaires and in the databases. Actual identifiers (e.g. names) will not be written on the header of the questionnaire and will not be entered in any computer. The same identifier code will be used on laboratory specimens, questionnaires and consent forms (using pre-printed stickers or bar codes).
- To ensure the return of results to individuals, full identifying data will be written on the consent forms that will then be kept, together with a study inclusion register that has the names and study identifiers, under lock and key under the responsibility of the principal investigator. These consent forms will be destroyed and/or handled as per data protection regulations after completion of the study and return of test results to individuals.

Benefits to individual participants

- Investigators will ensure that participants can access the results of their tests within a reasonable period of time [to be determined].
- In case RDTs are used, return of test results will be immediate (to the individual or the individual's parents for children) and under circumstances that allow confidentiality. Adequate post-test counselling will be provided.
- In the case of laboratory-based in vitro diagnosis, the return will take place after a few weeks. All participants will be able to call a number to access their results using, for example, the identifier code. However, individuals with positive test results will be actively followed up by investigators to organize referral for assessment of the need for care and treatment.

Informed consent

- Upon arrival in a household, trained field enumerators will briefly introduce themselves and the authority on behalf of which they are conducting the study.
- Participants (or their parents) will be fully informed about the risks and benefits associated with participation. They will sign a written informed consent form and/or document their consent through other means in case of illiteracy or young age (assent).
- If a participant cannot read, a relative from the same household may serve as a witness and sign on behalf the participant who is unable to read.
- In case of cluster sampling: upon arrival at the pre-selected household, the surveyor will invite all eligible household members to participate. Eligible persons (or their parents or the household head) will be asked for consent. If more than one person in the household needs to provide consent, then the informed consent information will be read to all individuals simultaneously with the opportunity to ask questions. It is also possible that one person has to provide consent for themselves and their child(ren). They will be asked to provide consent individually for themselves and their children.
- Children will be read an information sheet written in simplified language and requested to sign a dedicated assent form testifying their assent to the questionnaire and blood collection. Children under 6 years of age will not be asked to assent for blood draw because their maturity level precludes their ability to understand the assent procedure.
- Participants will be asked during the consent process if they agree to the storage of their blood specimens for other potential testing as deemed of public health relevance by the Ministry of Health. This is optional. Because specimens will be anonymised (link between patient and unique identifier broken) at the end of the study, the results of any future testing will not be able to be returned to the participant. *[Delete what does not apply.]*
- See Appendix 1: Template consent form for the participants of a viral hepatitis biomarker survey.
- See Appendix 2: Template assent form for children participating in a hepatitis B and C biomarker survey.

Expected benefits of the survey

- Patients identified with HBV or HCV infection during the biomarker survey will be referred for care.
- If a statistical sample has been used, the biomarker survey will generate reliable, population-based estimates of the prevalence of biomarkers of viral hepatitis.
- These estimates will provide information about the status of the country and where it stands with relation to achieving regional and global control/elimination goals.
- These estimates will be used as input parameters for modelling work to estimate the current burden due to present infection and to appraise various options for prevention, testing and treatment.
- The estimates could provide evidence of the need to establish a national plan for control/elimination of viral hepatitis along with associated services (e.g. vaccination, infection control, prevention, care and treatment).
- These estimates could guide the formulation of a national plan for control/elimination of viral hepatitis along with associated services (e.g. vaccination, infection control, prevention, care and treatment).

Roles and responsibilities

- List all participating institutions along with their roles and responsibilities.

Institution name	Contact person	Phone and email of contact person	Key role and responsibility

Reporting

- A written report of the survey will be prepared within 4 months of completion of testing.
- Investigators will organize a debriefing session of the results with all stakeholders to ensure that the information is used.
- The principal investigator will prepare a manuscript for submission to a peer-reviewed journal.
- Authorship will be decided on the basis of international criteria and a description of the contributions.

Timeline

- All procedures associated with the biomarker survey will be planned according to a timeline (see Appendix 12: Template timeline for a hepatitis biomarker survey).
- All tasks will be planned as per a GANTT chart to make sure that all the steps follow each other smoothly (e.g. reserving a session with the ethics committee in advance).

Budget

- The budget will follow the estimate as per Appendix 13: Template budget for a hepatitis biomarker survey.

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APPENDICES

Appendix 1: Template consent form for participants of a viral hepatitis biomarker survey

Title: [Insert official name of the biomarker survey]

Survey/Principal Investigator: [Insert name, title and contact information]

Survey sponsor: [Insert name of the institution or implementing agency/ies]

Survey overview

You have been chosen by chance to take part in a survey on hepatitis in [name of the country]. Hepatitis is a disease of the liver that is caused by a virus. The objective of this survey is to assess how common hepatitis viruses are in [name of country], including [spell out viruses involved]. On the basis of the results of the survey, the Ministry of Health could plan better services for prevention, testing and treatment.

I will tell you all you need to know before you decide to take part or not in the survey. It involves a few questions and a blood test. It's up to you whether you want to take part or not. As we go through this information please ask questions about whatever is not clear. We will be happy to provide any clarification.

If you agree to take part and sign the consent form, you will receive a copy of this form. By signing, you won't be giving up any legal rights. You can change your mind at any time, without giving reasons. You can also skip any questions that you do not wish to answer. If you refuse to take part, we will leave you alone from now on, and your decision will not affect the health-care services you or your family receives through the Ministry of Health or the public health system. We will not ask more questions and we will not do any blood tests.

Procedures

This survey will take about XX minutes of your time. First, we will ask you a set of questions intended to describe your situation, things that you could be doing that would put you at risk for these diseases, and your relevant history. Also, we would like to take a little bit of your blood (approximately XX mL) [specify the technique]. This blood will be tested for infection with viral hepatitis [specify the viruses].

Risks and discomforts

The risks are very small; however, taking blood may cause some discomfort, bleeding, bruising and/or swelling at the blood draw site. In rare cases, there is also a risk of fainting or an infection. In case you develop any problem as a result of our study, we would provide adequate treatment or make arrangements for treatment at health facilities, and will bear all costs related to the treatment. It is also possible that talking about your personal situation during the survey may make you feel uncomfortable.

Will I receive the results of any tests? [adapt if RDTs are used]

After [specify the number of days], you will be able to receive the results of your test. To obtain the results of your test, you may call this phone number [XXX-XXX-XXX] and provide your secret identification number: _____. However, if you happen to test positive for hepatitis, health-care workers will get in touch with you to make sure you get medical attention. We will then give you all the information that you will need for your next steps in terms of free medical care and assist you with the process. If you wish to know whether you have [name of the virus] infection but do not want to participate in this survey, you could go to [XXXX] and discuss with the doctors about testing.

What if some blood is left after the test?

If any blood is left over after the hepatitis test, it will be destroyed.

What benefits will I receive?

If you take part in the survey, this will greatly assist the public health system in [name of the country]. In addition, the survey will give you the benefit of knowing if you have any of these infections. If you are found positive, we will help you to protect others from the disease and assist you so that you can be treated. We will refer you to the appropriate treatment facilities; the arrangements will be as follows [explain how things will work].

Will I be paid?

Although payment will not be provided for your participation, we will provide your test results free of charge.

Confidentiality

Data collected from this survey will be available only to the [name of the institution] and [possible partners], who are assisting with survey implementation and data analysis. These agencies will keep all survey records private. Your name will not be used on any survey record on any computer. Instead, we will use a unique number that does not identify you personally. When we present or publish the survey results, we will not make reference to your name. The key to the code will be kept under lock and key.

Voluntary participation and withdrawal from the survey

You have the right to stop taking part in this survey at any time without penalty. You may refuse to take part in any step you do not feel happy with. You can also refuse to answer any questions that you do not wish to answer. You may withdraw from the survey at any time, and doing so will in no way affect your access to health-care services or drugs.

Contact information

Contact [title/name of the PI/institution] about this study or your part in it, or if you have questions or concerns about the survey.

Contact [title/name of a contact from the ethics committee/institution] about this study or your part in it, or if you have questions or concerns about the ethical aspects of the project.

Consent

Please print your name and sign below if you agree to take part in this survey. By signing this form, you will not give up any of your legal rights. We will give you a copy of the signed consent form to keep.

Last name: _____ **First name:** _____

Date of birth: _____ [Day] _____ [Month] _____ [year]

Address: _____ **City/Village:** _____ **Landmark:** _____

Cell phone: _____ **Phone:** _____

Unique identifier: _____

Signature of participant

Date (DD/MM/YY) _____

Assent for children

Signature of witness

Date (DD/MM/YY) _____

Consent for illiterate participants

Signature of witness

Date (DD/MM/YY) _____

Appendix 2: Template assent form for children participating in a hepatitis B and C biomarker survey

Survey/Principal Investigator: Phone number:
Survey sponsor:

Part 1: Information sheet

Introduction

My name is _____. I work for to understand how common certain diseases are among people in [Country X]. We are interested today in two diseases: hepatitis B and hepatitis C.

I will explain what we are doing and ask you to be in a research study. It's about taking your blood and finding out if you have hepatitis. We will not give you money for that. We have talked to your parent(s)/guardian and they know that we are also asking you. Before deciding, you can take advice from all the persons you want. After that, you can tell me if you want to take part or not. You do not have to decide right now. You can also change your mind whenever.

Purpose

You have been picked by chance to be in this study on hepatitis. Hepatitis is a disease of the liver. For hepatitis B, there is an effective vaccine to protect against catching the disease. For hepatitis C, there is no vaccine but there is an effective treatment to cure people who are sick. We do this study to count how many people are sick with hepatitis B and hepatitis C. The results of this survey will help us to protect people better.

What is going to happen

This study will take about 15 minutes. First, we will ask you some questions and find out which vaccines you have received. We would then like to take a bit of your blood [quantity to be specified according to the test(s) performed] by pricking a vein. This blood will be tested for hepatitis B and C in the laboratory.

Risks, discomforts and benefits

Taking blood is a bit painful. It's like a mosquito bite. It can cause a little bleeding, bruising and/or swelling. If we do this blood test, we can find out if you have hepatitis B or C. If you have hepatitis B or hepatitis C, the people from the health centre will come and tell you so that you can get treated. No one else will find out; the results will be given to you and to your parents/guardians only.

I have checked with the child and she/he understands the risks, discomfort and benefits _____(initial).

Contact information

If you have questions, you can contact Dr [name PI]. Phone number: _____

<p>Phone number to call for results : _____</p> <p>Your unique number as a child: ___/___/___</p>

Part 2: Certificate of assent

I understand that the research is about hepatitis, that I will answer a few questions and provide blood, and that if I have hepatitis, I will be informed and offered care.

I have read this information (or had the information read to me), I have had my questions answered and I know that I can ask questions later if I have some.

- **I agree to take part in the research.**

OR

- **I do not wish to take part in the research and I have not signed the assent below. _____ (initialled by child/minor)**

Only if child assents:

Print name of child _____

Signature of child: _____

Date (day/month/year): _____

If illiterate child:

A literate witness must sign (if possible, this person should be selected by the participant, not be a parent, and should have no connection with the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness (not a parent) _____ AND Thumb print of participant

Signature of witness _____

Date (day/month/year): _____

Appendix 3: Template questionnaire for biomarker survey participants

Questionnaire for children (participants aged less than XX years)

A:		Field work information			Observations
Date					
Team ID					
Item	Variable name	Question	Response options	Skip pattern	Observations
B.		General information			
1	IDNUMBER				This will link with the identifying information on the consent form and on the laboratory specimen.
2	GENDSUBJ	What is the gender of the child?	1. Male 2. Female 3. Transgender 7. Don't know 8. Refuse	N/A	
3	DOB	What is the child's date of birth?	dd/mm/yyyy	N/A	
4	COUNBIRT	What is the child's country of birth?	_____ Country	N/A	
5	DISTRESI	What is the child's district of residence?	_____ District	N/A	
6	MINOSTAT	Does the child's belong to any of the following population groups?	1. Group 1 2. Group 2 3. Group 3 4. Group 4 7. Don't know 8. Refuse	N/A	This is for minorities or indigenous populations

C. COLLECTION OF INFORMATION ON VACCINATION FOR CHILDREN					
7	BIRTLOC	Where was the child born?	1. At home 2. In a primary health care facility 3. In a hospital 4. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination
8	BIRTFACI	Did a skilled assistant deliver the child?	1. Yes 2. No 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination Skilled birth attendant=someone with specialized training such as a midwife, nurse, doctor
9	VACCCARD	Do you have a card where (NAME)'s vaccinations are written down? IF YES: May I see it please?	1. Yes, seen 2. Yes, not seen 7. No card 8. Refuse	Skip to #11 if 2, 7 or 8	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination
10	VACCDATE	According to the card, what are the dates on which hepatitis B vaccine was received?	DOSE 1 [dd/mm/yyyy]: DOSE 2 [dd/mm/yyyy]: DOSE 3 [dd/mm/yyyy]: DOSE 4 [dd/mm/yyyy]:	N/A	Leave blank if dates are missing Only for hepatitis B biomarker surveys to evaluate the impact of vaccination
11	BIRTHDOSE	If no vaccination card seen: did the child receive hepatitis B vaccine?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #section D if 2, 7 or 8	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination
12	BIRTDODATE	When did the child receive the first dose?	dd/mm/yyyy	N/A	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination
13	VACCDOSE	How many doses of hepatitis B vaccine did the child receive in total?	____ Doses	N/A	Only for hepatitis B biomarker surveys to evaluate the impact of vaccination

D.	RESULTS OF RAPID DIAGNOSTIC TESTS				
14	RDTHEPB	Result RDT for hepatitis B	1. Positive 2. Negative 3. Not interpretable 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys using rapid diagnostic tests (RDTs)
15	RDTHEPC	Result RDT for hepatitis C	1. Positive 2. Negative 3. Not interpretable 7. Don't know 8. Refuse	N/A	Only for hepatitis C biomarker surveys using RDTs

Questionnaire for adults (participants aged XX years and above)

A:		Field work information			Observations
Date					
Team ID					
Item	Variable name	Question	Response options	Skip pattern	Observations
B.		General information			
1	IDNUMBER				This will link with the identifying information on the consent form and on the laboratory specimen.
2	GENSUBJ	What is the gender of the participant?	1. Male 2. Female 3. Transgender 7. Don't know 8. Refuse	N/A	
3	DOB	What is your date of birth?	dd/mm/yyyy	N/A	
4	COUNBIRT	What is your country of birth?	___ Country	N/A	
5	DISTRESI	What is your district of residence?	___ District	N/A	
6	MINOSTAT	Do you belong to any of the following population groups?	1. Group 1 2. Group 2 3. Group 3 4. Group 4 7. Don't know 8. Refuse	N/A	This is for minorities or indigenous populations
7	EDUCLEVE	How many years of schooling have you completed?	___ Years	N/A	

C.		Immunization			
8	EVERHEPA	Have you ever received hepatitis A vaccine?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #10 if 2, 7 or 8	Only for hepatitis A biomarker surveys
9	HEPASHOT	How many shots of hepatitis A vaccine did you receive?	__ Doses	N/A	Only for hepatitis A biomarker surveys
10	EVERHEPB	Have you ever received hepatitis B vaccine?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #12 if 2, 7 or 8	Only for hepatitis B biomarker surveys
11	HEPBSHOT	How many shots of hepatitis B vaccine did you receive?	__ Doses	N/A	Only for hepatitis B biomarker surveys
D.		Knowledge of status			
12	EVERTESB	Have you ever been tested for hepatitis B?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #16 if 2, 7 or 8	Only for hepatitis B biomarker surveys
13	LASTTESB	When did you have your most recent hepatitis B test?	1. Last 12 months 2. 1–5 years 3. >5 years 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys
14	RESUTESB	What was the result of your last hepatitis B test?	1. Positive 2. Negative 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys

15	HEPBMED	Are you currently taking medicine to treat hepatitis B?	1. Yes 2. No 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys
16	EVERTESC	Have you ever been tested for hepatitis C?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #20 if 2, 7 or 8	Only for hepatitis C biomarker surveys
17	LASTTESC	When did you have your most recent hepatitis C test?	1. Last 12 months 2. 1–5 years 3. >5 years 7. Don't know 8. Refuse	N/A	Only for hepatitis C biomarker surveys
18	RESUTESC	What was the result of your last hepatitis C test?	1. Positive 2. Negative 7. Don't know 8. Refuse	N/A	Only for hepatitis C biomarker surveys
19	HEPBMEC	Have you undergone hepatitis C curative treatment with interferon or direct-acting antivirals?	1. Yes 2. No 7. Don't know 8. Refuse	N/A	Only for hepatitis C biomarker surveys

E. PAST OR PRESENT HISTORY OF EXPOSURE					
20	HEALCARE	Are you a health-care worker* exposed to blood through patient care?	1. Yes 2. No 7. Don't know 8. Refuse	N/A	
21	EVERTRANS	Have you ever received a blood transfusion?	1. Yes 2. No 7. Don't know 8. Refuse	Skip to #23 if 2, 7 or 8	
22	TRANSDATE	When did you receive your first blood transfusion?	Year: _____	N/A	
F. RESULTS OF RAPID DIAGNOSTIC TESTS					
23	RDTHEPB	Result of RDT for hepatitis B	1. Positive 2. Negative 3. Not interpretable 7. Don't know 8. Refuse	N/A	Only for hepatitis B biomarker surveys using RDTs
24	RDTHEPC	Result of RDT for hepatitis C	1. Positive 2. Negative 3. Not interpretable 7. Don't know 8. Refuse	N/A	Only for hepatitis C biomarker surveys using RDTs

* Doctor, nurse, nursing aid or janitor

Appendix 4: Template household log sheet for hepatitis biomarker survey

Today's date:		Interviewers' names:																		
Department or province:		Enumeration area/cluster #:																		
HH #	Contact (Y/N)	Age groups eligible in household (HH)															# visits	Location, telephone # for follow up		
		0-4 years				5-9 years				10-15 years				>15 years						
		# in HH	Consent (Y/N)	Interview completed (Y/N)	Serum/RDT/DBS (Y/N)	# in HH	Consent (Y/N)	Interview completed (Y/N)	Serum/RDT/DBS (Y/N)	# in HH	Consent (Y/N)	Interview completed (Y/N)	Serum/RDT/DBS (Y/N)	# in HH	Consent (Y/N)	Interview completed (Y/N)	Serum/RDT/DBS (Y/N)			
1																				
2																				
3																				
4																				
5																				
6																				
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19																				

Appendix 5: Recommendations for pre- and post-test counselling for viral hepatitis*

The 5 Cs of testing

Participants in the survey will be tested according to the following guiding principles.

1. Consent

People being tested for viral hepatitis must give informed consent to be tested and counselled. Verbal consent is sufficient, and they should be informed of the process of testing and of their right to decline. Provision of information about testing and the need for consent can be delivered in a group setting, such as group health education, but clients should give consent in an individual and private manner. Health workers should carefully explain how a client can decline testing and ensure that no one coerces a client into being tested, and each person has a private opportunity to opt out of testing.

2. Confidentiality – ensuring a confidential setting and preserving confidentiality

Testing must be confidential, meaning that what the provider and the client discuss will not be disclosed to anyone else without the expressed consent of the person being tested. Confidentiality applies not only to the test results and report of hepatitis status but also to any personal information, such as information concerning sexual behaviour and the use of illegal drugs. Hepatitis testing services should avoid practices that can inadvertently reveal a client's test results to others in the waiting room or in the health facility. Experiences with HIV testing services have shown that a lack of confidentiality discourages people from using the testing services. Health workers and others who provide testing may need special training and sensitization regarding the confidentiality of medical records. Although confidentiality should always be respected, it should not be allowed to reinforce secrecy, stigma or shame. Counsellors should discuss, among other issues, whom the person may wish to inform and how they would like this to be done. Shared confidentiality with a partner or family members and health-care providers is often highly beneficial.

3. Counselling

Pre-test information can be provided in a group setting, but all people should have the opportunity to ask questions in a private setting if they request it. All hepatitis testing must be accompanied by appropriate post-test counselling, based on the specific hepatitis test result and hepatitis status reported. Quality assurance mechanisms as well as supportive supervision and mentoring systems should be in place to ensure the provision of high-quality counselling.

4. Correct

Providers of hepatitis testing services should strive to provide high-quality services, and quality assurance mechanisms should ensure that people receive a correct diagnosis. Quality assurance may include both internal and external measures, including support from national or regional reference laboratories. All people who receive a positive serological diagnosis of HBV or HCV infection should undergo a NAT to confirm the presence of viraemic infection and assess their need for care and treatment before starting on antiviral therapy.

5. Connection

Linkage to prevention, treatment and care services should include effective and appropriate follow up, including long-term prevention and treatment support. Providing viral hepatitis testing where there is no access to care, or poor linkage to care and treatment, has limited benefit for those with hepatitis.

Pre-test information for hepatitis B and C serosurveys

Pre-test information will be delivered to the household once an agreement to participate has been obtained.

Clear and concise information should be given covering the following points:

- There are two tests to be conducted; the test for hepatitis B and the test for hepatitis C.
- One usually acquires hepatitis B or hepatitis C infection through exposure to blood (e.g. through receiving an injection with a dirty needle) or body fluids.

* From Guidelines on hepatitis B and C testing. Geneva: WHO; 2017.

- Hepatitis B and C cannot be acquired through casual daily contacts such as shaking hands or sharing food.
- Viral hepatitis B and C are chronic diseases that can affect the liver for a long time in a silent way. After years of silent presence, the liver can suffer some scarring or cancer. These complications can lead to death.
- Hepatitis B is preventable with a vaccine. An individual needs three doses to be fully protected.
- The test results will be returned to the person tested and to no one else.
- The test conducted allows detection of the virus in the blood.
 - If it is negative, it means that the virus is not present.
 - If it is positive, it means that the virus is present and that there is a risk of liver complications after many years. When the virus is present, there are a number of things that can be done to reduce the risk of developing complications. In some cases, treatment is necessary. In others, follow up is indicated.

Post-test information

Health workers will provide post-test counselling in a way that will be tailored to the unique situation of each individual. Key elements will include the following:

For those who test positive for hepatitis B

- The test results suggest that there is hepatitis B virus in the blood. It means that the virus is likely to be present and could harm the liver in the long term.
- To understand the effect of the virus on the liver, more tests need to be done, including a test to measure how much virus is in the blood and a test to see if the virus has damaged the liver.
- These tests will be organized as per the following plans [*explain*].
- As the hepatitis B virus is in the blood, there is a possible risk that others could have been infected, including close friends, sexual partners and family members. The way others could have been infected is through exposing them to blood, through having sex or through giving birth. Hence, we recommend that family members and sexual partners be vaccinated to prevent any risk of transmission. It would be a good idea to have them also tested for HBV infection as they could have been infected in the past.
- To prevent further damage to the liver, alcohol consumption may need to be limited (a few questions on drinking habits should help evaluate if such reduction is needed). Eating too much should also be avoided to limit overweight and regular physical activity encouraged.
- If there is a regular sexual partner, having a conversation with that person to inform him or her of the situation could be considered so that he or she gets vaccinated against hepatitis B (three doses required to be fully protected). Help can be provided for this process.

For those who test positive for hepatitis C

- The test results suggest that there has been contact with the hepatitis C virus at some stage. There are two options. The virus could still be present and could harm the liver in the long term, or the virus may have gone away by itself already.
- To understand better, more tests need to be done, including a test to check if the virus is still in the blood and a test to see if the virus has damaged the liver.
- These tests will be organized as per the following plans [*explain*].
- If the hepatitis C virus is confirmed to be still in the blood, there is a very low risk that others could have been infected, including close friends, sexual partners and family members. The way others could have been infected is through exposing them to blood, through having sex or through giving birth. Hence, it would be a good idea to have them also tested for HCV infection as they could have been infected in the past. No vaccine is currently available to protect your close contacts.
- If infection with hepatitis C virus is confirmed, to prevent further damage to the liver, alcohol consumption may need to be limited (a few questions on drinking habits should help evaluate if such reduction is needed). Eating too much should also be avoided to limit overweight and regular physical activity is encouraged.
- If there is a regular sexual partner, having a conversation with that person to inform him or her of the situation could be considered. However, the risk of sexual transmission is very low. Hence, there would not be any need

to change anything in your behaviour. Help can be provided for the process of talking to your sexual partners about the situation.

Additional referrals

Additional referrals will be provided, if needed, for prevention, counselling, support and other services, as appropriate. These could include, for example, HIV, TB, STI diagnosis and treatment, contraception, antenatal care, reducing alcohol use, opioid substitution therapy, access to sterile needles and syringes, and brief safe-sex counselling.

For those who test negative

- Individuals who test negative will not actively be sought to be given their results. However, they will be able to call a phone number and obtain the results upon provision of an identification number and answering a security question. They will just be told the results of their test. No counselling will be provided for these individuals. Families of children will already have been counselled to complete the vaccine series, if not completed.

Appendix 6: Dummy table shell for the analysis of a viral hepatitis biomarker survey, [location], 20XX*

Characteristics		Hepatitis B and D													
		Hepatitis A		Hepatitis E		Hepatitis B				Hepatitis D		Hepatitis C			
		Serological evidence of past or present HAV infection (anti-HAV+)		Serological evidence of past or present HEV infection (anti-HEV+)		Serological evidence of past or present HBV infection (anti-HBc+)		Chronic HBV infection (HBsAg+)		Chronic HDV coinfection (HDV)		Serological evidence of past or present HCV infection (anti-HCV+)		Chronic HCV infection (HCV RNA+)	
		(N=XXX)		(N=XXX)		(N=XXX)		(N=XXX)		(N=XXX)		(N=XXX)		(N=XXX)	
		#/Total	%	#/Total	%	#/Total	%	#/Total	%	#/Total	%	#/Total	%	#/Total	%
Age (years)	0–14	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	15–29	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	30–59	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	60+	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
Sex	Male	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	Female	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
Regions	North	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	East	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	South	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	West	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	Central	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
Specific populations	HCW	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%
	Minority	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%	XXX/XXX	XX%

anti-HAV: antibodies against hepatitis A virus; anti-HEV: antibodies against hepatitis E virus; anti-HBc: antibody against hepatitis B core antigen; anti-HCV: antibody against hepatitis C virus; HBV: hepatitis B virus; HCV: hepatitis C virus; RNA: ribonucleic acid

* Age groups, geographical areas and/or specific populations would need to be defined on the basis of the local epidemiology and history of the vaccination programme. Note that if the sample is not self-weighting, weight adjustment may be needed for percentages.

Appendix 7: Dummy table shells to compare groups with different characteristics in terms of prevalence of viral hepatitis biomarkers

Prevalence of HBV and HCV infection among [add study population] by exposure, in [add year] in [add geographical area]

		Prevalence among unexposed		Prevalence among exposed		Measure of association	
		#/Total	%	#/Total	%	Prevalence ratio	95% CI
HBV infection	Health-care work	XXX/XXX	XX%	XXX/XXX	XX%	XX%	XXX/XXX
	Ever transfused	XXX/XXX	XX%	XXX/XXX	XX%	XX%	XXX/XXX
HCV infection	Health-care work	XXX/XXX	XX%	XXX/XXX	XX%	XX%	XXX/XXX
	Ever transfused	XXX/XXX	XX%	XXX/XXX	XX%	XX%	XXX/XXX

Prevalence of HBsAg among [add study population] by vaccination status*, in [add year] in [add geographical area]

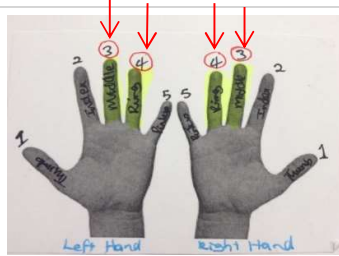
		N total	N HBsAg+	% HBsAg+	95% CI	P-value
Received any Hep B birth dose	Yes					
	No					
Age at which Hep B birth dose was received	<=1 day					
	>1 day					
	not received					
Received timely birth dose and 3 doses of pentavalent vaccine	Yes					
	No					
Received any birth dose and 2 or 3 doses of pentavalent vaccine	Yes					
	No					

*[Specify how the vaccination status was ascertained.]

Appendix 8: Job aid for collection of capillary blood



1. Ask client to rub/massage hands together. Ensure the client's hands are warm to touch.



2. Position hand palm-side up. Choose least calloused finger (either middle or ring finger).



3. Apply intermittent pressure to help the blood flow.



4. Disinfect fingertip. Start in the middle and work outward to avoid contamination. Allow to dry.



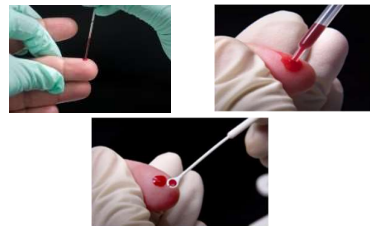
5. Hold the finger and firmly place a new sterile lancet off-centre on the fingertip.



6. Firmly press the lancet to puncture the fingertip.



7. Wipe away the first drop of blood with a sterile gauze pad or cotton ball.



8. If necessary, apply intermittent pressure on opposite side of finger for blood to flow.



9. Apply a gauze pad or cotton ball to the puncture site until the bleeding stops.



10. Properly dispose of all contaminated supplies. Do not retrieve anything from the waste containers.

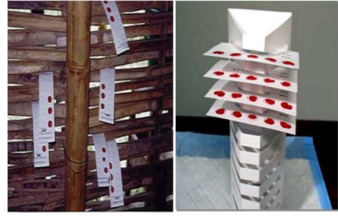
Appendix 9: Job aid for preparation, handling and storage of dried blood spots



1. Follow finger-prick procedure.



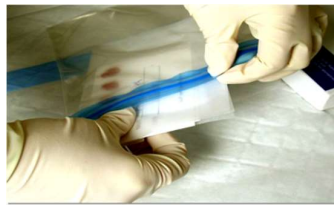
2. Apply blood to card using a specimen collection device or by allowing a free-falling drop from the finger onto the card.



3. Dry completely (at least 2 hours) before packaging.



4. Place filter paper between sheets of weighing paper.



5. Insert into sealable plastic bag.



6. Add desiccants and humidity cards and seal bag.

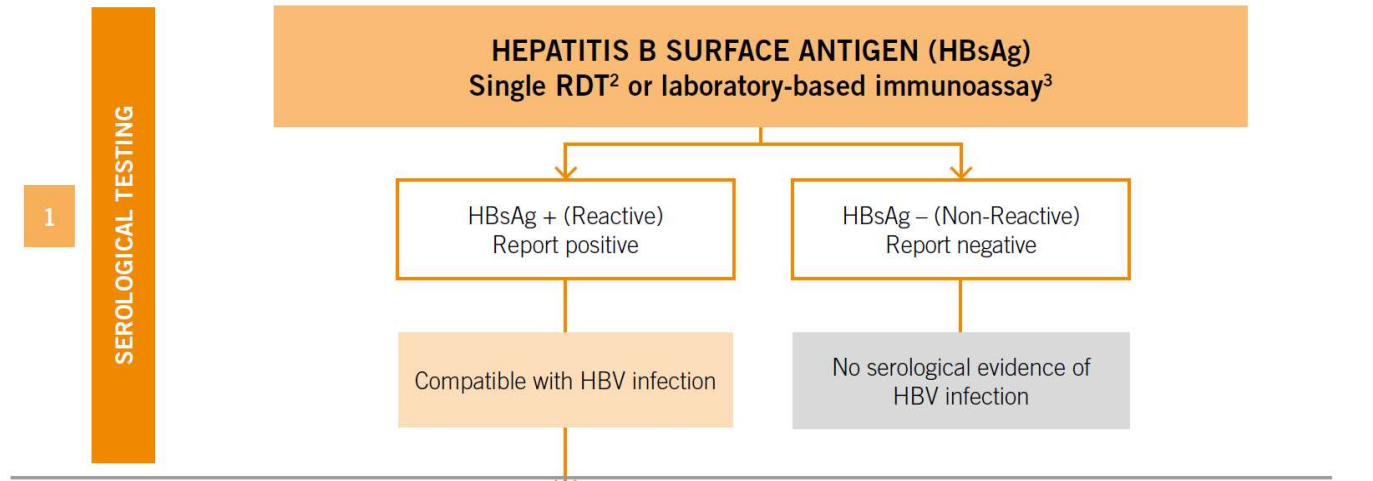


7. Organize DBS specimens according to specimen IDs.



8. Before shipment insert bundled DBS specimens and appropriate documentation into rip-resistant envelope.

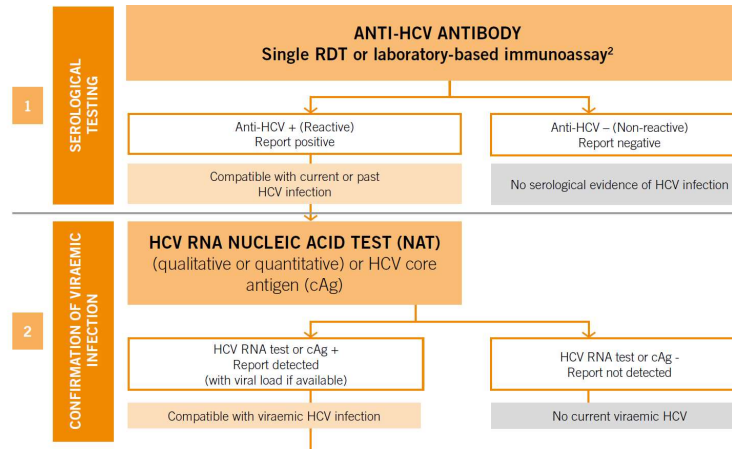
Appendix 10: Testing strategy for the diagnosis of HBV infection in biomarker surveys



2. In settings or populations with a low HBsAg seroprevalence (<0.4%), confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered.

3. Laboratory-based immunoassays include enzyme immunoassay (EIA), chemoluminescence immunoassay (CLIA) and electrochemoluminescence assay (ECL).

Appendix 11: Testing strategy for the diagnosis of HCV infection in biomarker surveys



². Laboratory-based immunoassays include enzyme immunoassay (EIA), chemoluminescence immunoassay (CLIA) and electrochemoluminescence assay (ECL).

Appendix 12: Template timeline for a hepatitis biomarker survey

Elements of workplan	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Protocol preparation	Concept paper	First draft	Revised draft	Approved version	Ethics committee review	Finalized version	Documentation of changes					
Training and logistics preparation				Development of training material	Ordering supplies for blood collection							
Field work					Pilot-testing questionnaire	Training of field workers	Field work	Field work	Debrief			
In vitro diagnostics				Test procurement	Test ordering	Test delivery	Preparations	Testing		Reporting		
Management of participants							Test returned <i>(as batches are operated or immediately for RDTs)</i>				Patients' assessment	
Data analysis							Data entry and data cleaning			Data merging	Data analysis	Writing the first draft of the report

Appendix 13: Template budget for a hepatitis biomarker survey

Item	#	Unit cost	Duration/ quantity	Total	Amount covered by investigators	Amount covered by other sources	
						Amount	Source(s) of funding
1. Enumeration of households (including training) [only if probability cluster sampling]							
Per diem (for all staff, including local guides and drivers)							
Lodging							
Training material							
Vehicle and fuel							
Other (e.g. phone cards)							
Subtotal enumeration							
2. Data collection (including training)							
Per diem (for all staff, including local guides and drivers)							
Lodging							
Training (venue rental, catering)							
Supplies for field implementation (e.g. bags, stationery)							
Vehicle and fuel							
Subtotal data collection							
3. Data entry and analysis							
Data entry and data cleaning							
Data analysis							
Report writing							
Subtotal data entry and analysis							
4. Laboratory supplies and testing							
Blood collection supplies*							
Blood collection supplies							
For RDTs							
Contact-activated lancets							
Capillary tubes							
Chase buffer							
RDT for HBsAg, anti-HCV, etc.							

* To be adapted, depending on type of test used: serology/DBS or RDT. May include alcohol, needle and tube holder, serum separation tube, gauze squares, cotton balls, band aid, bleach wipes, sharps container, absorbent sheet, gloves, biohazard waste bag, nalgene cryovial, cryo box, portable centrifuges, cool boxes, cool packs, bar code sheets.

For DBS							
Filter paper							
Drying rack							
Desiccant							
Humidity indicator							
Plastic bag							
For serological and molecular tests (according to objectives)							
Laboratory specialists							
HBsAg							
HBV DNA							
Anti-HCV							
HCV RNA							
Anti-HAV							
Anti-HEV							
Subtotal lab supplies and testing costs							
5. Case management of participants testing positive							
Confirmation testing for those testing HBsAg+							
HCV RNA for those testing anti-HCV+							
Staging testing for those testing HBsAg+							
Fibrosis assessment for those testing anti-HCV+							
One year of HBV treatment for those eligible for treatment							
Curative HCV treatment for those testing HCV RNA+							
Subtotal case management of positives							
6. Other costs (to be adapted according to context)							
Ethics Committee protocol review							
Insurance for field teams							
Repair of vehicles as needed							
Shipment of specimens to national, regional or reference labs							
Quality control testing for specimens at reference laboratory							
Presentation of results							
Subtotal other costs							
Total of 1, 2, 3, 4, 5 and 6							
Administrative costs							
TOTAL budget							

Appendix 14: Tool for rapid assessment of laboratory capacity before a viral hepatitis biomarker survey

Viral hepatitis laboratory: preliminary assessment	
Laboratory location, leadership and affiliation	
Country	
Town	
Date	
Name of the laboratory	
Name of the director	
Name of the respondent	
Position of the respondent (director, deputy director, technician, unit director, etc.)	
Level of laboratory (peripheral, intermediate, reference)	
AFFILIATION: public health/private or hospital/health centre/veterinary/other	
Qualification and number of laboratory staff	
High level (MD, PhD, MS, MPH)	
Technician (MT)	
Janitors	
Number of viral hepatitis tests performed during the year before this assessment	
Total anti-HAV	
IgM anti-HAV	
Total anti-HBc	
Anti-HBs	
HBsAg	
HBV DNA	
Anti-HCV	
HCV RNA	
Number of equipment units and consumables	
Autoclave	
Water purification system	
Clinical centrifuge, 3000 rpm	
Incubator	
Freezer –70° (optional)	
Freezer –20°	
Refrigerator (4–8°C)	
Disposable gloves	
Disposable lab coats	
Disposable pipette tips	
Disposable serological pipettes	
Computer + printer	
Internet connection (1-year subscription)	
ELISA plate washer	
ELISA plate incubator (37 °C)	
ELISA plate reader (capable of reading at 405 nm, 450 nm, 490 nm and reference at 630 nm)	
Automatic pipettors P10+	
Automatic pipettors P100+	
Automatic pipettors P1000	
Multichannel automatic pipettors P10+	
Multichannel automatic pipettors P100+	
Multichannel automatic pipettors P1000	
Pipette aid (for serological pipettes)	
Timer	

Does the laboratory have the following standard operating procedures for a quality management system, approved by the laboratory director and quality manager? (Yes/No)	
Pre-analytical phase	
Test request	
Test selection	
Trained testing personnel	
Patient/client preparation	
Specimen collection, labelling and transport	
Analytical phase	
Specimen processing and storage	
Reagent preparation	
Preventive maintenance/equipment checks	
Quality control	
Test performance	
Proficiency testing/external quality assessment	
Specimen storage	
Post-analytical phase	
Reviewing quality control	
Transcribing results	
Reporting results	
Maintenance of records	
Laboratory accreditation and external quality assurance (EQA)	

ISO 9001 2015

ISO 15189 2012

If there is an accreditation body, please specify

Does the laboratory participate in an external quality assurance programme? (Yes/No)

Appendix 15: User guide for the template protocol

Introduction

Biomarker surveys are the reference method to estimate the prevalence of any infection with the hepatitis viruses. Prevalence estimates are useful in a number of contexts.

- **Hepatitis B and C viruses.** The prevalence of hepatitis B virus/hepatitis C virus (HBV/HCV) infection is the first core indicator of the monitoring and evaluation framework of hepatitis elimination. It is key to the initial assessment phase.
- **Hepatitis B virus and immunization.** When seroprevalence surveys are conducted after implementation of universal immunization for infants against HBV, the results can be used for impact assessment. Biomarkers of other hepatitis viruses could be included as needed.
- **Hepatitis D virus.** If there are elements suggesting that the frequency of hepatitis D virus (HDV) coinfection is of public health importance among HBV-infected patients, such patients could be tested for biomarkers of HDV infection.
- **Hepatitis A virus.** In countries with intermediate hepatitis A virus (HAV) endemicity and no vaccination programme against HAV, the prevalence of antibodies against HAV by age group, along with information on the reported rates of hepatitis A, may help to guide decision-making on possible inclusion of hepatitis A vaccine in the routine immunization schedule ⁽¹⁾.
- **Hepatitis E virus.** If the epidemiology of hepatitis E virus (HEV) infection is unclear, the prevalence of antibodies to HEV by age group may help to interpret data on reported rates of acute hepatitis E and guide interventions for prevention and control.

This user guide aims to assist survey implementers to adapt the template protocol to their local setting, section by section.

Target audience

The target audience for this protocol template includes viral hepatitis programme managers, immunization programme managers, primary investigators of viral hepatitis biomarker surveys and epidemiologists/public health officials who may provide technical assistance to national officials for the development of a protocol for conducting a viral hepatitis biomarker survey.

Objective

The objective of the tool is to provide a template that can be adapted for developing a protocol for a viral hepatitis biomarker survey.

Guidance already available that may be used to prepare for a hepatitis biomarker survey

The template of a protocol is an application of guidance that is already available in the following documents.

1. **Guidelines for HIV biomarker surveys in the general population ⁽²⁾**
These UNAIDS/WHO guidelines explain how to conduct population surveys to evaluate the impact of response to a national HIV epidemic. All basic principles in these guidelines, particularly in terms of sampling, would apply to a hepatitis survey.
2. **Biobehavioural survey guidelines for populations at risk for HIV: reference manual ⁽³⁾**
These WHO/UNAIDS guidelines explain how to conduct surveys in key populations to evaluate the impact of the response to the HIV epidemic among them.

3. **Guidance for biomarker surveys to evaluate the impact of universal hepatitis B immunization (4)**

This WHO document explains how to conduct a population biomarker survey in children to evaluate the impact of universal hepatitis B immunization.

4. **Guidance for sampling approaches for biomarker surveys to evaluate the impact of universal hepatitis B immunization (5)**

This WHO document further details the sampling aspects of biomarker surveys to evaluate the impact of hepatitis B immunization.

5. **WHO vaccination coverage cluster surveys: reference manual (6)**

This WHO manual explains the different steps in conducting cluster surveys while ensuring that probability sampling is respected.

Use of the template protocol in practice

To generate a survey protocol, users of this document may proceed as follows:

1. Review previous biomarker surveys conducted locally, understand the local epidemiological situation and find out about existing/available technical resources.
2. Review key WHO guidance documents on biomarker surveys (mentioned above).
3. Review this template to become familiar with the key elements.
4. Organize a meeting of national stakeholders to obtain answers to the key questions that need to be addressed (see below).
5. Adapt this template protocol to the local situation.
6. Seek formal peer-review comments.
7. Submit to the ethics committee(s)^a to apply for clearance.
8. If possible, provide feedback to WHO regarding the usefulness of this tool.

Multiple options in this template protocol

The protocol often proposes more than one option [delete what does not apply]. When this is the case, options are listed from the most preferable to the least preferable.

Questions to address to adapt this template into a national protocol

In this section, the user will answer a series of questions that will help in selecting the appropriate sections to keep in the protocol.

To decide on the study objective(s), users need to start by clarifying the public health actions that may be guided by the survey. Table 1 in the template protocol includes examples of public health questions that may be addressed by biomarker surveys.

Title

The title must include the biomarkers tested, the population, timing and geographical area.

Investigators

Include all significant contributors along with their roles and responsibilities, and cite funding sources.

^a The protocol needs to be submitted to all ethics committees as per the institutes/facilities involved in the survey. Permission from the national data protection agency may be required.

Background

This section should include general information about viral hepatitis and country information about the local epidemiology of viral hepatitis (major risk groups), previous seroprevalence study/expected prevalence, vaccination schedule and coverage of hepatitis B and A vaccines, testing and linkage-to-care strategy and policies that will be guided by the results of this study.

Objectives

- Study objectives need to address the public health policies under consideration.
- Exposures of interest (e.g. geographical area, sex, etc.) based on the local epidemiology and public health programmes in place can be considered as secondary objectives.
- For age group categories, planning on reporting by 5-year age group bands will align the results with international publications for future meta-analyses.

Epidemiological methods

Population

The population needs to be defined according to the study objectives and may include exclusion criteria.

Design

- A biomarker survey is a cross-sectional study.

Synergies

- The synergies section explains with which (if any) other survey this biomarker survey will be combined.
- A sufficient quantity of blood specimens will need to be collected.
- The advantages of combining surveys should be balanced against different specific logistic constraints, as well as practical considerations (e.g. ethical clearance/stigma/diagnostic algorithm/linkage to care).

Sampling procedures

In the sampling procedure approach, the preferred approach is the use of a simple random sample (Fig. 1). However, this requires an individual sampling frame of the entire study population. Furthermore, randomly selecting and interviewing individuals one by one at the national level may be time- and resource-consuming. A more convenient alternative approach would be cluster surveys, where geographical clusters of individuals are selected all together. This allows the investigation teams to go to fewer places and save time and resources. In the case of cluster sampling, depending on the study population (e.g. specific age group), it may be more efficient and less prone to bias to include all eligible individuals in the study, depending on household composition and the size of the enumeration areas (if small). If the survey focuses exclusively on school-age children, clusters can be schools and/or classrooms. Note that convenience sampling is unacceptable for the purposes of verifying that a country has achieved a regional hepatitis B control goal in children.

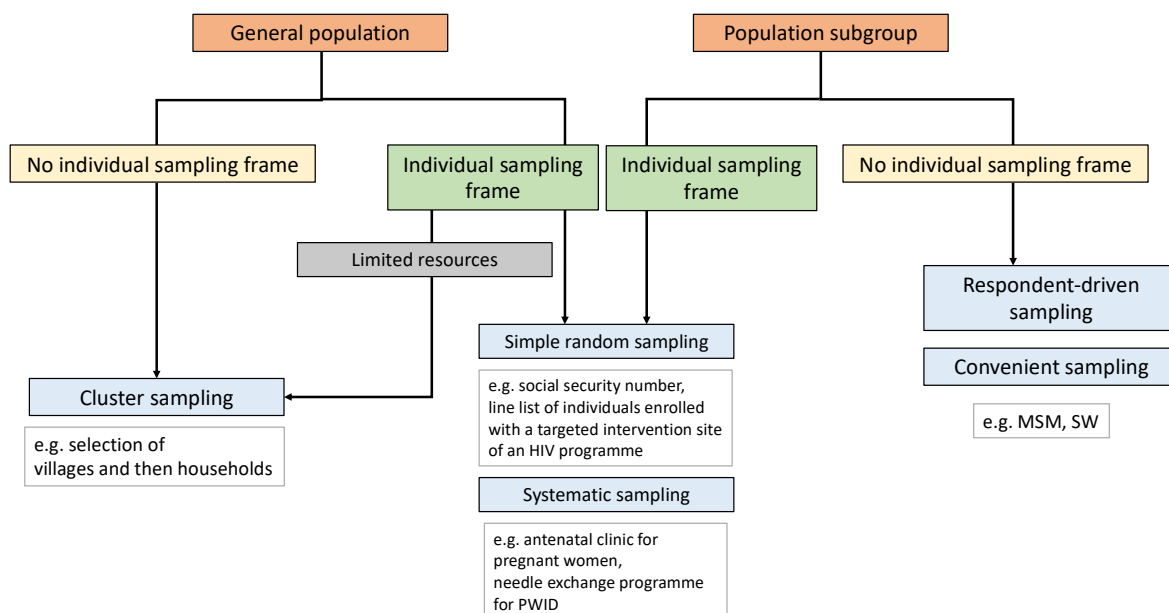


Fig. 1: Choice of an appropriate sampling procedure

MSM: men who have sex with men; PWID: people who inject drugs; SW: sex worker

Sample size calculation

- Use a sample size calculator such as www.openepi.com.
- Decide on the level of precision required for the estimates based on the expected prevalence, study objectives and public health actions to be guided.
- Use pre-existing data or regional estimates to define parameters to be used to calculate the minimum sample size.
- Table 1 in the template protocol can be used to examine the precision that would be obtained for various biomarkers (e.g. hepatitis B surface antigen [HBsAg], anti-HCV). A workable compromise could be made to decide on a sample size that will give the required precision for the prevalence estimates of the biomarkers considered within acceptable time and budget limits.

Data collection

- Plan the tasks to be conducted by each team member. Usually, teams include one interviewer, one laboratory technician or nurse, and one supervisor. However, the size and composition of field teams may depend on the sample size to be achieved, the size of the areas to be covered, laboratory tests to be used and resources available.
- Identify a logistics supplier who can handle storage and shipment of blood specimens in the public health system or among partners.
 - Data collected:
 - Select relevant questions according to the study objectives.
 - Specify how children and adults are defined.
 - Additional items may be needed according to the sampling procedure used. For cluster surveys, this would include the cluster number and the household number. For respondent-

driven sampling, this would include the identifier of the recruiter (see participant leading to this individual in the template protocol). For systematic sampling in the health facility, this may include the name of the health facility.

- To decide on the data collection tool, take into consideration:
 - amount of data to be collected (number of questions/questionnaires);
 - resources available to buy data collection devices, develop data collection software and enter data (if paper-based field data collection);
 - security issues;
 - access to power;
 - computer literacy of the field team;
 - availability of mobile data connection in the field.

In vitro diagnostic methods

A decision on which diagnostic methods (Table 1) to use should be based on the study objectives and biomarkers needed, study population, field constraints and cold chain requirement (Table 2), financial and human resources, and available WHO prequalified tests (for a list of WHO-prequalified tests see http://www.who.int/diagnostics_laboratory/evaluations/PQ_list/en/).

Rapid diagnostic tests (RDTs) for the detection of antibodies usually need no more than 50 µL specimen (usually more capillary whole blood volume required than serum/plasma) whereas RDTs for the detection of antigen may need up to 100 µL (capillary whole blood protocol usually requires more volume than serum/plasma). Manually loaded enzyme immunoassays (EIAs) require up to 100 µL. Instrument-based immunoassays may require up to 1 mL of serum/plasma. RDTs that are currently commercially available cover HBsAg, hepatitis B e antigen (HBeAg), anti-HCV, anti-HAV and anti-HEV.

In the WHO testing guidelines, the quality of evidence for the use of **dried blood spot (DBS)** specimen-based assays has been ranked as low to moderate for serological and nucleic acid testing of HCV and HBV. DBS should be used only in conditions that preclude better performing tests, as described in Box 1. DBS results should be returned to the patients only if the assays used have been validated for use on DBS.

Oral fluid-based tests (e.g. Oraquick for HCV) are increasingly becoming available and represent a promising option for future surveys.^b However, in the absence of field experience at this stage, the template focuses exclusively on blood specimens. If oral fluid-based tests are to be used, the protocol needs to be adapted according to the manufacturer's instructions and the tests conducted as per intended use.

Specimen processing, storage and transport

Blood specimens should be stored and transported in containers separate from any vaccine products or medicines.

In vitro diagnosis

Ensure that the testing strategies follow the WHO testing guidelines for viral hepatitis.

^b OraQuick HCV rapid antibody test is WHO prequalified, including for oral fluid. The current instructions for use state that the performance claim made for sensitivity is 98.1% (95% CI: 96.9–99.0%), which has been verified by WHO. The specificity is 99.6% (95% CI: 99.2–99.9%). These are lower than for capillary whole blood, venous whole blood, plasma and serum but are still acceptable.

Box 3: WHO recommendations on the use of DBS specimens for testing individuals for HBV or HCV infection (7)

a) Serology

The use of DBS specimens for HBsAg and anti-HCV antibody serology testing^c may be considered in settings where:

- cold chain during the transport of specimens or their storage cannot be ensured;
- there are no facilities or expertise to take venous whole blood specimens; or
- RDTs are not available or their use is not feasible; or
- there are persons with poor venous access (e.g. PWID) and RDTs are not feasible.

b)

c) Nucleic acid testing (NAT)

The use of DBS specimens to test for HBV DNA and HCV RNA for diagnosing chronic HBV and HCV infection, respectively, may be considered in settings where:

- cold chain during the transport of specimens or their storage cannot be ensured;
- there is a lack of access to sites or nearby laboratory facilities for NAT, or provision for timely delivery of specimens to a laboratory; or
- there are persons with poor venous access (e.g. those in drug treatment programmes, prisons).

Analysis plan

- When possible, use double data entry to limit errors. If resources are limited, double data entry can be limited to essential information (e.g. identifiers and laboratory results).
- Prepare a plan for descriptive analysis to identify implausible data and clean the dataset. To limit data entry errors, add checks to the data entry mask.
- In the analysis plan, take into account the sampling design and weights.
 - In the case of **cluster sampling**, refer to *WHO vaccination coverage cluster surveys: reference manual*.⁶
 - In the case of a **respondent-driven sampling** approach, useful resources are available on <http://www.respondentdrivensampling.org/>.

Quality assurance

- Describe what is planned to ensure good-quality work at all stages of the study, from the data and specimens collected in the field to their processing and analysis.

Limitations

- List all the potential biases that may affect the study results.

Safety and monitoring

An adverse event can be any unfavourable and unintended sign (including an abnormal laboratory finding^d), symptom or disease temporally associated with the performance of a protocol-specified procedure, whether or not considered related to the procedure itself. Normal physiological changes that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events.

A "serious adverse event" (SAE) means an adverse event that is fatal or life-threatening, results in persistent or significant disability/incapacity, requires inpatient hospitalization or prolongation of existing inpatient hospitalization, or results in a congenital anomaly/birth defect, cancer, is a consequence of an overdose or is

^c There are currently no in vitro diagnostics that are validated by their manufacturers for use with DBS specimens, nor regulatory approval for use of DBS specimens. Therefore, use of DBS specimens would be considered "off-label".

^d An adverse event for diagnostics is defined as a product defect (i.e. malfunction or failure, deterioration in characteristics or performance, or inadequacy of labelling or of instructions for use) that, directly or indirectly, might lead to or might have led to serious medical consequences, namely, death or serious deterioration in the state of health of the patient, user or another person.

another important medical event. This for the most part would not apply to a biomarker survey. The only procedure being performed is acquisition of a blood specimen, which carries a minimal risk of SAEs.

Linkage to care

- Adapt the framework to the local health-care system.
- Describe how pre-counselling as well as counselling and treatment of patients testing positive will be organized.

Protection of human subjects

Community engagement and follow up

Describe how the team will engage the community to prepare the field and feed the results back to the community.

Risk/inconvenience to individual participants

Describe what the participants may endure during data collection and how the confidentiality of the interview and the anonymity of the information collected will be ensured.

Benefits to individual participants

Explain how the laboratory results will be fed back to the patients and how individuals testing positive will benefit from their participation by being offered treatment.

Informed consent

Prepare a separate assent form for children with simplified language and that can be used for them to assent to the questionnaire and blood collection (example provided in Appendix 2 of the template protocol).

Expected benefits of the survey

This section lists individual and public health benefits from the survey. It includes plans to use the results to guide public health action ⁽⁸⁾.

Budget

The total budget for the survey should include field costs, in vitro diagnosis and treatment for those testing positive. The source of funding should be planned for all these subcomponents of the budget. In countries where the national health insurance takes care of treatment, the survey budget can mention that treatment will be supported by the national health insurance.

References

- ¹ WHO. WHO position paper on hepatitis A vaccines: June 2012 – recommendations. *Vaccine*. 2013;31(2):285–6.
- ² UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance 2015. Guidelines on monitoring the impact of the HIV epidemic using population-based surveys. Geneva: UNAIDS; 2015 (http://www.unaids.org/en/resources/documents/2015/population_based_surveys, accessed 21 November 2018).
- ³ Biobehavioural survey guidelines for populations at risk for HIV. Global HIV Strategic Information Working Group. WHO: Geneva, Switzerland; 2017 (available at <http://apps.who.int/iris/bitstream/handle/10665/258924/9789241513012-eng.pdf?sequence=1&isAllowed=y>, accessed 17 May 2018).
- ⁴ Documenting the impact of hepatitis B immunization: best practices for conducting a serosurvey. Geneva: WHO; 2011 [WHO/IVB/11.08] (http://apps.who.int/iris/bitstream/10665/70808/1/WHO_IVB_11.08_eng.pdf, accessed 15 November 2018).
- ⁵ Sample design and procedures for hepatitis B immunization surveys: a companion to the WHO cluster survey reference manual. Geneva: WHO; 2012 [WHO/IVB/11.12] (http://apps.who.int/iris/bitstream/10665/70876/1/WHO_IVB_11.12_eng.pdf, accessed 15 November 2018).
- ⁶ WHO vaccination coverage cluster surveys: reference manual. Version 3 (July 2015). Geneva: WHO; 2015 (http://www.who.int/immunization/monitoring_surveillance/Vaccination_coverage_cluster_survey_with_annexes.pdf, accessed 15 November 2018).
- ⁷ Guidelines on hepatitis B and C testing. Policy brief. Geneva: WHO; 2016 [WHO/HIV/2016.23] (<http://apps.who.int/iris/bitstream/10665/251330/1/WHO-HIV-2016.23-eng.pdf?ua=1>, accessed 15 November 2018).
- ⁸ Hutin Y, Low-Beer D, Bergeri I, Hess S, Garcia-Calleja JM, Hayashi C, et al. Viral hepatitis strategic information to achieve elimination by 2030: key elements for HIV program managers. *JMIR Public Health Surveill*. 15 déc 2017;3(4):e91 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5747598/>, accessed 18 May 2018).

Table 1: Advantages and disadvantages of different assay formats

Assays	Advantages	Disadvantages
Laboratory-based immunoassays (EIA, CLIA, ECL)	<ul style="list-style-type: none"> • Currently superior clinical/diagnostic and analytical sensitivity/specificity for HBsAg • High throughput possible (>40 per day per operator) • High throughput increases further when using automated immunoanalysers • Objective, automated reading of results, but not for line blots or simple assays • Within-assay procedural quality control 	<ul style="list-style-type: none"> • Requires laboratory facilities, equipment, e.g. EIA plate washers, readers, incubators or immunoanalysers or random access analysers • Requires trained laboratory technician • Reagents require refrigeration • Requires venepuncture to obtain specimen • Time to result ~3 hours and generally batched as one run if manual EIA
Rapid diagnostic tests (RDTs)	<ul style="list-style-type: none"> • Accessible at the lowest level of the health-care system (including community settings) • Does not specifically require laboratory facilities • May be carried out by trained lay providers and health-care workers, as well as laboratory technicians • Can be used with less invasive specimens that do not require venepuncture such as capillary whole blood or oral fluid • If testing at or near the point of care, same-day results are possible, which may reduce the number of individuals lost to follow up and therefore do not receive their test results • Devices can be stored at 2–30 °C 	<ul style="list-style-type: none"> • Lower clinical and analytical sensitivity/specificity for HBsAg • Less sensitive in certain populations such as immunosuppressed persons, including HIV-positive individuals • Ineffective within-assay quality control, i.e. most RDTs do not control for specimen addition • Lack of external control reagents for quality control of test kit with most RDTs, but some exceptions, e.g. Oraquick • Stability at room temperature is impacted by environmental factors, e.g. heat, humidity, storage conditions • Subjective reading and interpretation of results • Requires manual transcription of testing results into laboratory logbook/testing register, partially mitigated by automated RDT readers
Nucleic acid testing (NAT) technologies	<ul style="list-style-type: none"> • May be used at or near the point of care • May be carried out by trained lay providers and health-care workers, as well as laboratory technicians • Can be used with less invasive specimens that do not require venepuncture such as capillary whole blood • Devices can be stored at 2–30 °C 	<ul style="list-style-type: none"> • Currently requires laboratory facilities and equipment, but this may not apply to future point-of-care options • Requires trained laboratory technician • Reagents require refrigeration • Requires venepuncture to obtain specimen • Time to result ~3 hours and generally batched as one

Source: Guidelines on hepatitis B and C testing. Geneva: WHO; February 2017 (<http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1>, accessed 15 November 2018).

CLIA: chemiluminescent immunoassay; ECL: electrochemiluminescence; EIA: enzyme immunoassay; HBsAg: hepatitis B surface antigen; NAT: nucleic acid testing; RDT: rapid diagnostic test

Table 2: Specimen types and processing requirements

Specimen type	Time to processing/storage/time to testing
<p>Venous whole blood Fresh whole blood collected by venepuncture</p>	<ul style="list-style-type: none"> • Use the specimen immediately.
<p>Serum Freshly collected whole blood is allowed to coagulate, and the serum fraction is collected away from the clotted red blood cells.</p>	<ul style="list-style-type: none"> • Collect whole blood, mix by hand 4–5 times immediately and let stand for the clot to form. • Process within 30 minutes of collection. • Store at 2–8 °C. Test within 5 days or as specified by the instructions for the assay to be used.
<p>Plasma Freshly collected whole blood is added to the recommended anticoagulant, such as EDTA, heparin or citrate. After centrifugation, plasma is separated.</p>	<ul style="list-style-type: none"> • Collect whole blood, mix by hand 8–10 times immediately and centrifuge for up to 10 minutes. • Process within 6 hours of collection. • Store at 2–8 °C. Test within 5 days or as specified by the instructions for the assay to be used.
<p>Capillary whole blood Capillary (finger-stick) whole blood is collected using a lancet and a specimen transfer device.</p>	<ul style="list-style-type: none"> • Use the specimen immediately, with the specimen transfer device recommended by the instructions for use. • Note that the specimen transfer device may or may not include an anticoagulant. An anticoagulant contributes to accuracy.
<p>Oral fluid Oral mucosal transudate (not saliva) is collected from the gums using a collection device.</p>	<ul style="list-style-type: none"> • Use the specimen immediately, with the specimen transfer device recommended in the instructions for use.
<p>Dried blood spot (DBS) Venous or capillary whole blood is applied to a filter paper by hanging drop or microcapillary action. Whole blood is later eluted from the filter paper and used for the test procedure.</p>	<ul style="list-style-type: none"> • Store at 4 °C for up to 3 months, or at –20 °C for longer. • Use of specific assays with DBS should be validated by the manufacturer. If the manufacturer has not validated their assay for DBS, the use of DBS is considered “off-label”, or unauthorized for returning medical results.

Source: Guidelines on hepatitis B and C testing. Geneva: WHO; February 2017 (<http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1>, accessed 15 November 2018).