



# Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients



Centre Collaborateur de l'OMS  
pour la Recherche en Epidémiologie  
et la Réponse aux Maladies Emergentes

**Study protocol**

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## LIST OF ABBREVIATIONS AND ACRONYMS

Ab	Antibody
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-Retroviral Therapy
CD4	Cluster of Differentiation 4
CI	Confidence Interval
CRF	Case Report Form
DAAs	Directly Acting Antivirals (drug)
EIA	Enzyme Immunoassay
ERB	Ethics Review Board
HBsAg	Antigen anti-HBs
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ILBS	Institute of Liver and Biliary Studies
IU	International Unit
LMICs	Low and Middle Income Countries
LoA	Limit of Agreement
LR +/-	Positive/Negative Likelihood Ratio
mL	Milliliter
MoH	Ministry of Health
MSF	Médecins Sans Frontières (Doctors without Borders)
MSM	Men who have Sex with Men
µl	Microliter
OD	Optical density
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PLHIV	People living with HIV
PWIDs	People Who Inject Drugs
qPCR	quantitative PCR
RDT	Rapid Diagnostic Test
RIBA	Recombinant Immune Blot Assay
RNA	Ribonucleic Acid
RT-PCR	Real-Time PCR
Se	Sensibility
Sp	Specificity
USD	United States Dollar
VL	Viral Load
WHO	World Health Organization

- **Title:** Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients
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## PROTOCOL SUMMARY

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- **Title:** Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients.
- **Aim:** To evaluate the performance of serological HCV Rapid Diagnostic Tests (RDTs) to identify tests adapted to resource-limited settings, reliable for HIV-infected patients.
- **Study design:** Prospective evaluation of the performance of serological RDTs for HCV screening.
- **Primary objective:**
  - To evaluate the performance of serological HCV RDTs under field conditions using as reference standard a combination of enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA) for the detection of antibodies anti-HCV in HIV infected patients.
- **Secondary objectives:**
  - To describe the accuracy (sensitivity, specificity) of the RDTs as screening tests
  - To describe the performance of the RDTs according to the HCV genotype and HCV VL
  - To describe the performance of the RDTs according to the CD4 counts and HIV VL
  - To describe the performance of the RDTs in presence of HBV co-infection (presence of antigen anti-HBs (HBsAg))
  - To describe the operational characteristics of the tests including ease of use, technical complexity and inter-reader variability.
  - To evaluate predictive values of each HCV RDT based on the prevalence of the testing center.
- **Tests evaluated:** Six to seven HCV RDTs will be evaluated, including Oraquick HCV (Orasure, USA), HCV TriDot 4<sup>th</sup> (J.Mitra, India), SD Bioline HCV (Standard Diagnostics, Korea and India). The other tests will be selected based on an on-going dossier assessment.
- **Study Population:** HIV patients attending HIV clinics for their regular follow-up and fulfilling the inclusion criteria.
- **Methods:** Blood samples will be prospectively collected and used in field conditions to perform the different HCV RDTs under evaluation. In the reference laboratory, ILBS, the reference technique (EIA + RIBA) will be carried to ascertain the true serological status of each sample. Other tests will be performed to characterize the HCV infection (viral load and genotype), the HIV and HBV status of the participants.
- **Study duration:** The expected overall period for participant inclusion and tests evaluation is one year. The inclusion period may be slightly extended in order to reach the sample size.

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## DEFINITIONS

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- **Acute HCV Infection:** 6 month period following acquisition of hepatitis C virus.
- **Chronic HCV Infection:** Long lasting infection ( $\geq$  six months) without clear decrease or disappearance of the viral load.
- **Gold standard:** Referral technique to establish the true condition status.
- **Rapid Diagnostic Test (RDT):** An assay intended to provide diagnostic results conveniently, immediately to the patient while still at the health facility, screening site, or to other health care providers.
- **Sensitivity of a test (Se):** The ability of the test to correctly identify those patients with the disease (True Positives / (True Positives + False Negatives)).
- **Specificity of a test (Sp):** The ability of the test to correctly identify those patients without the disease (True negatives / (True negatives + False Positives)).
- **Negative predictive value** Proportion of negative diagnostic tests that are true negative.
- **Positive predictive value** Proportion of positive diagnostic tests that are true positive.

Predictive values are influenced by the prevalence of the disease in the population.

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# I. INTRODUCTION

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## 1.1 Hepatitis C infection

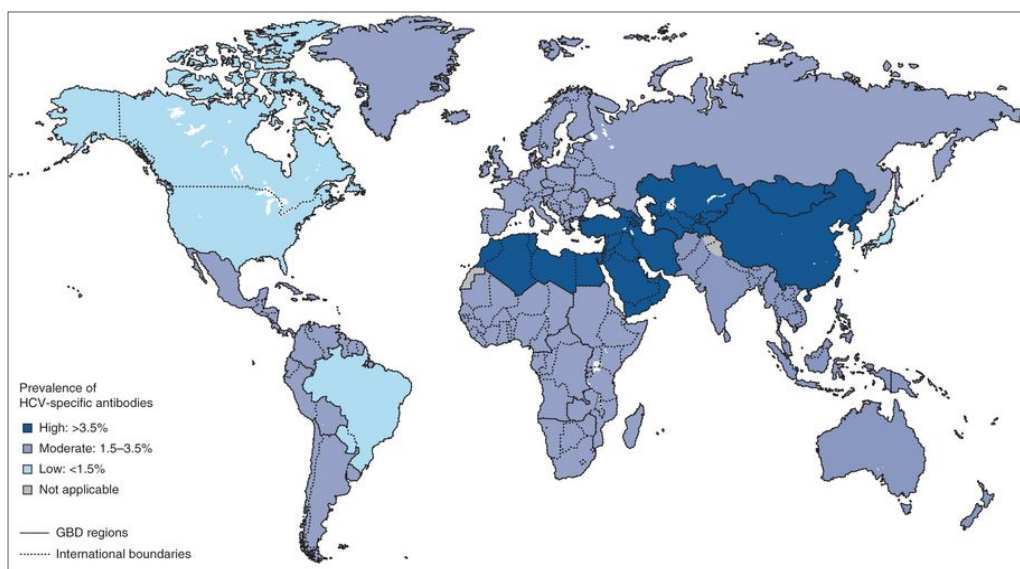
Hepatitis C virus (HCV) belonging to *Flaviviridae* family (1) is a blood-borne RNA virus that is commonly transmitted parenterally: through blood and its derivatives, invasive procedures in health care facilities with inadequate infection control practices and injections with contaminated needles (2). Sexual transmission is strongly linked to HIV pre-infection (2,3). Less common is the vertical transmission (from mother to child). Between 55 and 85% of reported HCV infections will evolve in chronic disease. From 10 to 15% of those who are chronically infected are estimated to develop liver cirrhosis depending on factors that accelerate fibrosis progression such as age, sex, alcohol consumption, HIV or HBV co-infection, and obesity (4,5). The risk of developing hepatocellular carcinoma for a patient with HCV-related cirrhosis is approximately 2-6% per year (6).

## 1.2 Epidemiology of HCV infection

Recent estimates indicate nearly 185 million people chronically infected with HCV, of whom about 350,000 - 500,000 die every year (7). This prevalence represents 3% of the worldwide population with an important geographic variability in the global distribution. Central - East Asia, North Africa and the Middle East are the most affected regions (2) (Fig.1).

In low and middle-income countries (LMICs), HCV infection is predominantly associated with unscreened blood transfusion or unsafe injection practices (2). In contrast, in middle and high-income countries, unsterile equipment used to inject drugs represents the main route of transmission (2).

Most people are unaware of being carriers of HCV. The majority of them live in resource-limited settings where there is currently little or no access to diagnosis and treatment (8). Due to the absence of reliable epidemiological data, disease prevalence rate, globally, remains uncertain and HCV-related mortality is likely underestimated (9).



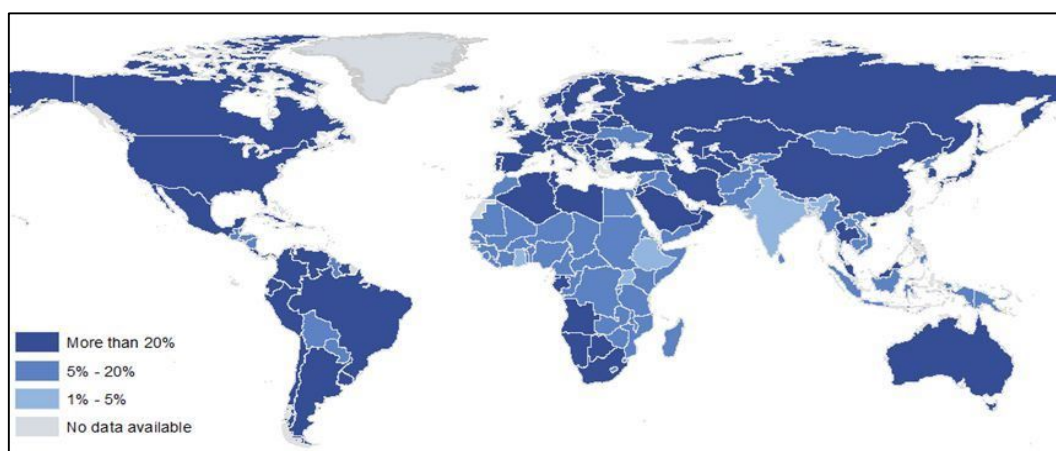
**Figure 1:** Prevalence of HCV-specific antibodies worldwide (source: Thomas DL, Nature Medicine 19, 850-3, 2013).

### 1.2.1 Epidemiology of HCV infection in India

Despite a low to moderate (1-1.5%) prevalence of HCV, India accounts for a large proportion of the worldwide HCV burden due to its large population: approximately 12-18 million people are infected (10). Population-based studies on HCV prevalence are scarce and most of data are based on blood bank screening (11) that presents some limitations. However, this data shows important geographic variability that may represent a realistic prevalence variation due to differences in cultural and healthcare practices in different regions, or variations in donor population or screening tests used (11). Different hotspots have been identified in the northeastern part of the country, Punjab, and in tribal populations where the prevalence may vary from 5.2% (in Punjab with a mixed urban and rural population) (12) up to 14.4% (in Bharia tribe) (13). Contrariwise, the lower prevalence is registered in western and eastern areas of the country. The predominant route of HCV transmission remains the parenteral one that occurs through unsafe therapeutic injections and blood transfusion. Injection drug use is limited to few pockets (11). There is a need for large field studies to better estimate the HCV prevalence, to identify the high prevalence areas as well as the associated risk factors.

### 1.3 HCV and HIV co-infection

The burden of HCV and HIV co-infection represents an increasing problem for the future. Between four to five million people living with HIV are estimated to be co-infected with HCV, representing 16% of people living with HIV (PLHIV) (14,15). As a result of common transmission routes, co-infection rates in people who inject drugs, men who have sex with men and hemophiliacs are especially high (60-90%) (16), (Fig. 2). HIV infection has a clinical impact on HCV although the pathogenesis of this interaction remains unclear. PLHIV are less likely to clear spontaneously HCV following infection and they experience a more rapid HCV disease (17). Furthermore, HIV infected individuals have higher HCV viral load (VL) that, consequently, would facilitate hepatic inflammation, even if this result has not been widely confirmed. Conversely, HCV infection has important repercussions on a more rapid progression to AIDS, higher levels of HIV virus in the blood, poorer control on ART (11% loss of ART efficacy in co-infected patients) and more common neurocognitive deficits (18–20). Co-infection with HCV in the era of anti-retroviral therapy (ART) is associated with worse HIV-related outcomes including weaker immune recovery (lower mean CD4 counts). Finally, co-infected individuals remain at a significantly increased risk of overall mortality when compared to their HIV mono-infected counterparts (21).



**Figure 2:** Percentage of HIV+ individuals with HCV infection by country (source: Center for Disease Analysis, Oral abstract: Prevention strategies for HIV/HCV infection by Isabelle Andrieux-Meyer, at IAS Kuala Lumpur, Indonesia, July 2013. With permission)

## 1.4 Diagnosis of HCV infection

### 1.4.1 Serological assays

Serological screening and diagnosis of HCV can be performed by using *indirect tests* which detect antibodies anti-HCV and *direct tests* (or *antigen assays*) which detect and may also quantify viral components.

Serological tests are realized by enzymatic immunoassays (EIA), recombinant immunoblot assays (RIBA), chemo-luminescence assays (CIA) or RDTs (based on agglutination, immune-chromatography or immune-filtration principle) (22). The EIAs detect a mixture of antibodies directed against various HCV epitopes. Different generations of EIAs have been developed, each one containing additional antigens in order to improve the sensitivity of the test. Currently the 3rd and 4th generations of EIA are commonly used in diagnostic laboratories. The specificity of this method is greater than 99% (23). Specificity is more difficult to establish due to the clearance phenomenon that characterizes the hepatitis C infection. However, it is recognized that it is excellent in immunocompetent people.

Immunoassays such as RIBA and strip immunoblot assays were developed to deal with false positives, particularly in low-prevalence settings. They are used as additional tests because of their higher specificity to confirm the presence of anti-HCV antibodies. However, in resource-limited settings, RIBAs have not been widely implemented due to their complexity, long turnaround time of test results and cost (24).

Today, the best reference method in terms of serological confirmation is given by the combination of EIA and RIBA assays. However, this combined approach is neither standardized nor used as routine. In practice, RIBA has been replaced by molecular tests that can directly confirm the active infection thus bypassing the serological confirmation. Nevertheless, this combination (EIA+RIBA) remains the standard for the WHO prequalification process of serological RDTs.

### 1.4.2 Molecular assays

Molecular assays based on nucleic acid testing (NAT), either *qualitative* or *quantitative*, give confirmation of a viremic infection. Qualitative assays (polymerase chain reaction, PCR) confirm the presence of the viral genome, providing a “yes/no” answer, while quantitative ones (Real Time-PCR or quantitative PCR, qPCR) monitor HCV RNA levels (viral load) in international units per milliliter (IU/mL). Qualitative molecular tests are cheaper, can be used for diagnosis and test of cure, whereas, HCV viral load (VL) testing is used to measure viral load burden and is more expensive than the qualitative one. However, both are rarely available and used primarily in specialized laboratories.

Access to those tests in LMICs is limited because of their complexity, high cost and logistic implications.

## 1.5 Poor access to HCV screening and diagnosis

Until recently, HCV was not recognized as a major public health problem. Lack of reliable epidemiological data on the burden of the disease and the absence of national and international policies have limited access to both diagnosis and treatment (25).

Globally, only 30% of the population has access to a free of charge HCV diagnosis (26). In LMICs, only 11% of infected people have access to HCV screening or diagnosis (8). Furthermore, the number of available, quality-approved and easy-to-use HCV tests, has remained limited.

The current continuum of testing for HCV and treatment monitoring is complex and expensive. The package of tests for screening, diagnosis, genotyping and monitoring costs around 500-600 USD per

patient (27). It is mainly laboratory-based and requires significant infrastructure and well-trained personnel. Hence, the provision of this diagnostic package is extremely limited in the LMICs. In settings with limited access to laboratory infrastructure, RDTs and POC tests delivered at health facilities level are recommended to improve access (28).

Currently, the existing HCV RDTs have showed limited accuracy in remote settings (29) and serological tests show poor accuracy in HIV co-infected populations (30,31).

Today, only one rapid serological test, the SD Bioline (Standard Diagnostics, Yongin, Korea) has been very recently pre-qualified by WHO (November 2016) and only one is FDA approved (OraSure, USA). The price of this latter (8 to 14 euros) is 4-12 times more expensive than other RDTs (27) and still precludes its scaling up. Thus, the paucity of qualified and well-performing tests maintains high prices and prevents competition among manufacturers thereby limiting their availability in LMICs.

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## II. STUDY RATIONALE

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Currently, a wide range of rapid diagnostic tests (RDTs) is available on the market, but these tests are characterized by variability in terms of performance (30) as showed in a systematic review conducted by Shivkumar and colleagues.

Despite the large offer, evaluation studies have been focused so far on very few RDTs. Accuracy estimates may be questioned for some studies due to the use of imperfect reference standards to ascertain the true disease status (30).

Furthermore, there is a paucity of data on the performance of HCV RDTs in patients having concomitant morbidity (e.g. HBV, HIV, syphilis). It is likely that the performance of serological tests is lower in case of HIV infection. The sensitivity of HCV testing may be still further reduced among persons with advanced immunosuppression due to HIV infection (32,33). Only few published articles mention the performance of HCV RDTs in HIV population. In addition, the evaluation of HCV rapid tests currently on-going for WHO prequalification does not include panels taken from HIV populations.

We propose to evaluate serologic HCV RDTs using samples from HIV infected patients followed in an MSF program in India.

This study aims to provide complementary and independent information on the performance of the selected tests in presence of HIV infection, in addition to the studies performed by the manufacturers. This study will contribute to identifying RDTs for HCV screening in PLHIV.

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## III. STUDY OBJECTIVES

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### 3.1 Primary objectives

To evaluate the performance of serological HCV RDTs under field conditions using as reference standard a combination of EIA + RIBA for the detection of antibodies anti-HCV in HIV infected patients.

### 3.2 Secondary objectives

- To describe the performance of the RDTs according to the HCV genotype and HCV VL.
- To describe the performance of the RDTs according to the CD4 counts and HIV VL.
- To describe the performance of the RDTs in presence of HBV co-infection (presence of HBsAg).
- To describe the operational characteristics of the tests including ease of use, technical complexity and inter-reader variability.
- To evaluate predictive values of each HCV RDT based on the prevalence of the testing center.

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## IV. STUDY DESIGN AND METHODS

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### 4.1 Study design

The study is a prospective evaluation of the performance of serological RDTs for HCV screening.

### 4.2 Sample size

As per WHO recommendations, the minimum performance criteria demanded for the Pre-Qualification (PQ) is 98% for both sensitivity and specificity.

The sample size is calculated based on the assumption that both Sensitivity (Se) and Specificity (Sp) are 98% and in order to provide a 95% confidence interval (CI<sub>95%</sub>) with a precision of  $\pm 2\%$  for both Se and Sp estimates. In order to obtain these estimates, a minimum of 190 HCV+/HIV+ samples and 190 HCV-/HIV+ samples are required.

With an expected 25% prevalence of HCV among PLHIV observed in the MSF HIV cohort in Manipur, a total of 760 HIV+ patients will be needed to reach the sample size. In addition, the sample size will be increased by 10% (836 total samples) to account for losses, problems in shipment, etc. The total number of patients needed to obtain sufficient HCV-positive patients will be recalculated during interim analysis. One hundred first samples will be sent, and the results of the reference standard will be used to re-estimate the sample size.

### 4.3 Study sites

Samples will be collected in three MSF HIV clinics in Manipur State in the northeastern India. The clinics are located in Churachandpur, Chakpikarong and Moreh.

A specific authorization will be asked by Epicentre and Institute of Liver and Billiary Sciences (ILBS) to MSF project in Manipur to have access to HIV patients followed in the MSF cohort.

Manipur is one of the six high prevalence states in India in terms of prevalence of HIV infection, contributing nearly 8% of India's total HIV positive cases (34). The catchment population of the three clinics is estimated around 415,000. By September 2014, in collaboration with the MoH, 1,404 patients were actively followed-up for HIV care, including ten patients on second line treatment. Regular screening for HCV was started at the end of 2010. In September 2014, the prevalence of co-infection found within the programs was 40% among 1,263 HIV-positive patients in Churachandpur and Chakpikarong and 20% among 814 patients screened in Moreh.

To develop the HCV project and ensure adequate screening of the patients, collaboration is developed with the national reference laboratory: ILBS in New Delhi. The reference methods will be performed in this laboratory as well as HCV VL, genotyping, HIV VL and hepatitis B serology.

#### **4.4 Study population**

HIV patients attending one of the selected clinics for their regular HIV follow up and fulfilling the inclusion criteria described below.

##### **4.4.1 Eligibility criteria**

Patients will be consecutively included in the study if they meet all of the following inclusion criteria and none of the exclusion criteria:

*Inclusion criteria:*

- Adults, aged  $\geq 18$  years
- HIV positive status confirmed according to the algorithm of the country
- Able and willing to provide signed informed consent

*Exclusion criteria:*

- Currently exposed to HCV treatment
- Inability to obtain a venous blood sample or insufficient blood (less than 10 ml of whole blood)
- Serious medical conditions not allowing additional blood withdrawal

#### **4.5 Duration of the study**

The overall period of participants' inclusion and tests evaluation is expected to be one year. The inclusion period (6 months) may be slightly extended in order to reach the sample size. The rhythm of participants' inclusion will differ according to site constraints and patients inflow.

#### **4.6 Study procedures**

##### **4.6.1 Participants enrolment and blood sample collection**

HIV patients regularly visiting the three selected facilities will be counselled concerning HCV and invited to participate to the study.

All participants meeting the inclusion criteria will be referred to the study staff (nurse or laboratory technician) who will explain the purpose of the study and will ask for written informed consent (Appendices A and B). If they agree to participate in the study, the study staff will collect 12 ml of whole blood.

If a patient refuses to participate, he/she will access the HCV care according to the MSF facilities procedures. Patients will be tested for HCV serology according to the test conducted in place (HCV TriDot 4<sup>th</sup>, J.Mitra, India).

#### 4.6.2 Laboratory procedures

The total volume of blood to collect is 12 ml (3x4ml EDTA tubes). The Table 1 displays the detailed use of blood drawn per participant.

	Test performed	Plasma volume/test (μl)	Plasma total (μl)	WB (μl)
<b>Field activity</b>	6 RDTs	5-50x6	300	600
	CD4			25
<b>ILBS activity</b>	EIA HCV	50	50	100
	RIBA HCV	50	50	100
	1 ELISA HBV	250	250	500
	VL HCV		1000	2000
	VL GT		1000	2000
	VL HIV		1000	2000
	backup volume			4675
	<b>Total</b>			12000

**Table 1:** Whole blood volume required for one patient according to the panel of tests.

The buffer stock will be used in case of handling, transport or storage problems to re-perform the necessary test.

RDTs may work on plasma/serum (P/S) or whole blood (WB). For RDTs working only on plasma/serum, the sample used will be plasma, for those working on plasma/serum and whole blood; whole blood specimens will be used.

##### Use of whole blood:

The RDTs that are compatible with whole blood will be evaluated using whole blood collected in the EDTA tube. Afterward, CD4 count will be done using PIMA system if the last available results are more than 3 months old.

##### Preparation of plasma /Aliquots

Plasma will be prepared by the study nurse or laboratory technician. Plasma will be used for RDTs whose intended use is plasma/serum only. Afterward the separated plasma will be transferred into 2 ml cryovials and frozen at -20°C. Every month samples collected will be shipped to ILBS, in New Delhi.

The workflow at each study site is described in the Appendix C.

#### 4.6.3 HCV RDTs

An array of 6 or 7 RDTs will be used to test all samples in the laboratory annexed to each MSF health center in the field. RDTs selection will be done considering their regulatory approval, the analytical performance, information available in literature and an on-going dossier review (ie. evaluation of the quality of manufacturing process). This list is not yet finalized and the final one will be provided once the dossier review will be finalized.

RDTs under evaluation will include at least the following tests:

- OraQuick HCV, OraSure, USA
- HCV TriDot 4<sup>th</sup>, J.Mitra, India
- SD Bioline HCV, Standard Diagnostics, Korea
- SD Bioline HCV, Standard Diagnostics, India



All tests will be performed according to manufacturer’s recommendations. Samples will be processed and read as showed in Table 2. Both laboratory technicians will read the test within the authorized time specified in the instructions for use.

	<b>RDT #1</b>	<b>RDT #2</b>	<b>RDT #3</b>	<b>RDT #4</b>	<b>.....</b>
<i>Samples processing</i>	Patient 1	Patient 1	Patient 1	Patient 1	
	Patient 2	Patient 2	Patient 2	Patient 2	
	Patient 3	Patient 3	Patient 3	Patient 3	
	Patient 4	Patient 4	Patient 4	Patient 4	
	Patient 5	Patient 5	Patient 5	Patient 5	
	<b>Lab tech 1</b>	<b>Lab tech 2</b>	<b>Lab tech 1</b>	<b>Lab tech 2</b>	
<i>Reading</i>	<b>Lab tech 1 and 2</b>	<b>Lab tech 2 and 1</b>	<b>Lab tech 1 and 2</b>	<b>Lab tech 2 and 1</b>	
<i>Samples processing</i>	Patient 6	Patient 6	Patient 6	Patient 6	
	Patient 7	Patient 7	Patient 7	Patient 7	
	...	...	...	...	
	<b>Lab tech 2</b>	<b>Lab tech 1</b>	<b>Lab tech 2</b>	<b>Lab tech 1</b>	
<i>Reading</i>	<b>Lab tech 1 and 2</b>	<b>Lab tech 2 and 1</b>	<b>Lab tech 1 and 2</b>	<b>Lab tech 2 and 1</b>	

**Table 2:** HCV RDTs samples processing and reading.

Technicians will report results in different laboratory registers.

In case of disagreement, a third reader will act as a tie-breaker, either the lab study coordinator or the site laboratory technician. In addition, a photo of all performed RDTs will be taken (eg: track of possible entry errors).

#### **4.7 Shipment and storage of samples to ILBS (New Delhi, India)**

For analysis, all collected samples will be sent every month to ILBS. The samples will be packed and transported in cold chain with ice packs to the ILBS in New Delhi. The shipment will be under the responsibility of the site investigator. Samples will be stored at -80°C at the ILBS.

#### **4.8 Procedures and analysis at the referral laboratory**

In the referral laboratory, reference assays and diagnostic tests will be performed:

- HCV and HBV EIAs and HIV viral load on all samples;
- HCV RIBA on all samples positive by EIA;
- HCV VL on all samples positive and indeterminate by RIBA;
- HCV genotyping on samples with a positive HCV viral load.

##### **4.8.1 Reference standard for HCV diagnosis**

###### **4.8.1.1 Enzyme immunoassay (EIA)**

All samples will be analyzed to detect the presence of anti-HCV Ab. For the HCV serology, the reference standard is given by an EIA followed by a recombinant immunoblot.

The commercial kit used for this purpose will be a 3<sup>rd</sup> generation EIA: Monolisa HCV Anti HCV plus version 2.0 (BioRad, Marnes la Coquette, France). It contains the antigens of nucleocapsid, NS3 and NS4 regions. Antibodies to core and NS3 regions are dominant at the point of seroconversion. Antibodies to NS4 and NS5 regions reach higher titers in the chronic phase of the seroconversion.

Test results will be interpreted according to the manufacturer’s instructions (Table 3):

Test name	Cut off definition	Results interpretation
Monolisa HCV Anti HCV plus version 2.0 (BioRad, Marnes la Coquette, France).	Mean value of negative control + 0.040 Optical Density (OD)	OD value of the sample < cut-off (non-reactive) OD value of the sample ≥ cut-off (reactive)

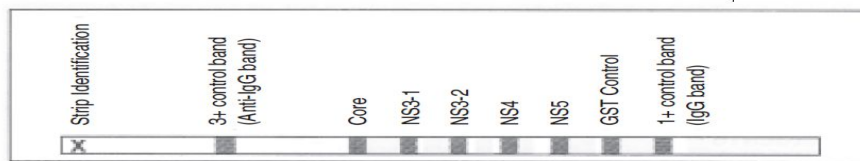
**Table 3:** EIA results interpretation according to manufacturer.

#### 4.8.1.2 Recombinant immune blot assay (RIBA)

The recombinant immunoblot used to complete the reference standard algorithm is MP Diagnostics HCV Blot 3.0. It utilizes nitrocellulose strips containing five recombinant HCV proteins from the capsid, NS3-1, NS3-2, NS4, and NS5 regions of the HCV genome. The HCV proteins are expressed as GST fusion proteins, so a GST control band is included to indicate reactivity to native GST. The blots also contain an IgG control band and an anti-IgG band. Individual nitrocellulose strips will be incubated with diluted serum or plasma specimens and controls. Specific antibodies to HCV, if present in the specimen, will bind to the HCV proteins on the strips.

The strips will be washed to remove unbound materials and then incubated with affinity purified anti-human IgG conjugated with alkaline phosphatase. The conjugate antibody will bind to any antigen-antibody complexes formed on the blots. Unbound conjugate will be removed by washing. A substrate will be added to visualize reactive protein bands on the blots.

The following (Fig. 3) is a diagram of the antigens and controls coated on MP HCV BLOT 3.0.



**Figure 3:** Diagram of the bands on the HCV BLOT 3.0 strip.

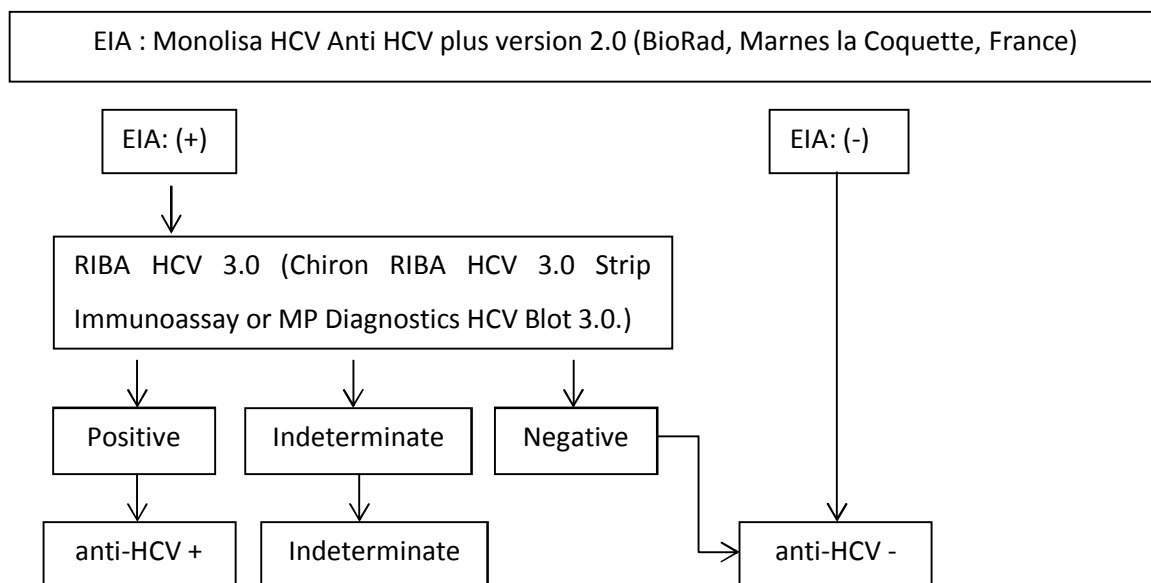
Once the washing is completed, it will be possible to locate and identify the intensity of the control bands. The 3+ intensity is the anti-IgG band and the 1+ intensity band is the IgG control band. These should be visible on all strips. The intensity of any reactive band will be compared to these two bands for reference. Comparison with these bands is performed in order to assign a reactivity rating to each antigen on the strip. The interpretation of the test is provided in the Table 4.

<b>PATTERN</b>	<b>INTERPRETATION</b>
1) No reactivity -	-
2) Reactivity < 1+ control	±
3) Reactivity = 1+ control	1+
4) Reactivity > 1+ and <3+ control	2+
5) Reactivity = 3+ control	3+
6) Reactivity > 3+ control	4+
<b>BLOT PROFILE</b>	<b>INTERPRETATION</b>
No bands of 1+ or greater reactivity	NEGATIVE
1+ or greater reactivity to 2 or more HCV antigens OR 2+ or greater reactivity to Core band only	POSITIVE
Any single HCV band of 1+ or greater reactivity but does not meet criteria for POSITIVE	INDETERMINATE

**Table 4:** Strip interpretation.

#### 4.8.1.3 Samples characterization

The algorithm for the characterization of samples will be as follows (Fig. 4):



**Figure 4:** Algorithm for characterization of samples.

Samples classified as anti-HCV positive and indeterminate will be further characterized by HCV viral load test. For all samples with a positive HCV VL, HCV genotyping will be done.

#### 4.8.2 HCV viral load and genotyping

HCV viral load will be performed on Abbott m2000® platform using Abbott RealTime HCV assay (detection limit: 12UI/mL) (Abbott Laboratories, Abbott Park, IL).

The HCV genotype determination will be performed for each HCV RNA positive sample. HCV genotyping will be performed by an in-house sequencing technique.

#### **4.8.3 HIV viral load monitoring**

All samples will be analyzed to quantify the HIV VL (if the last result is older than 3 months) using Abbott RealTime HIV assay (Abbott Laboratories, Abbott Park, IL).

#### **4.8.4 HBV diagnosis**

All samples will be tested for the presence of HBsAg indicating the presence of a HBV infection. The HBsAg will be detected by Abbott HBsAg qualitative Architect System (Abbott Laboratories, Abbott Park, IL).

### **4.9 Confirmatory results and treatment**

Results of the reference standard and results of HIV VL, HBsAg will be communicated and counselled according to care guidelines on site.

Following site guidelines, patients having a detectable HCV VL will be treated with the new generation of HCV drugs (DAAs) available on site. For HBV positive patients, HIV treatment will be adapted accordingly.

### **4.10 Evaluation of the RDTs Ease of Use**

Tests selected for the evaluation will be assessed for ease of use by laboratory technicians performing the test (questionnaire).

RDTs will be assessed according to the following elements:

- Clarity of kit instructions
- Technical complexity
- Ease of interpretation of results

A score will be calculated to reflect the ease of use.

The questionnaire will be administered to each lab technician twice during the study: at the beginning and at the end of the study.

### **4.11 Quality Assurance**

During the study, in addition to the pre-existing quality control in the reference laboratory (ILBS), a further external quality assurance will be performed. The quality assurance will consist of a proficiency testing prior to study implementation and during the study.

For this proficiency testing, panels of characterized samples will be sent by a French national referral laboratory (Institute National de Transfusion Sanguine, INTS, France) in order to ensure the quality of the results on the three techniques: serology, viral load and genotyping.

#### **4.12 Storage of remaining samples**

After the tests evaluation, the remaining samples will be kept at ILBS. Samples may be used to evaluate further HCV tests still under development. Furthermore, they might be used in case of treatment failure or relapse. Storage of the samples will be ensured for five years. At the end of this period, the remaining samples will be destroyed following internal laboratory standard procedures and national regulations. Samples will remain under the responsibility of the principal investigators (PI). ILBS will ensure proper management and communication regarding samples. All the evaluations not planned in this protocol will be approved by the PIs and by the scientific committee (if the study is still ongoing) or by the sponsor if the study is finished. A specific submission to the MSF Ethics Review Board (ERB) and the National Ethics Committee will be done.

#### **4.13 Training**

Before the beginning of the study, all study staff will receive appropriate training by Epicentre staff members and experienced consultants.

A specific training concerning the informed consent will be given to ensure the staff in charge respects the adequate procedures.

Only trained and competent staff will participate in the study.

The performance of the HCV tests might be influenced due to the lack of compliance with standard operating procedures (SOPs). Therefore, a monitoring of good practices of performing HCV test will be conducted regularly after the initial training and according to the field procedure in place. The field study coordinator will carry out this evaluation using a standardized checklist. According to these assessments, adapted and regular trainings will be conducted.

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## **V. DATA MANAGEMENT AND ANALYSIS**

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### **5.1 Data collection tools and data management**

For each study participant, all data collected during the study will be recorded in two standardized forms.

Thus, the Case Report Form (CRF) will be formed by:

- the patient information: age, sex, enrolment site, HIV treatment
- the laboratory tests results

The forms will be adapted to the evaluated tests. The reading is tests specific.

This is to ensure that the results collected follow the instruction of use and to facilitate review by supervisor.

The laboratory results will include:

- The field results: RDTs, CD4
- ILBS results : EIA and RIBA results, HCV Viral Load, HCV Genotype, HIV VL and serology  
HBsAg

The dates of the tests will also be recorded.

The information will be linked using a unique identification number. All the data will be coded and entered into an electronic database at each study site using electronic software.

The photographs of the RDTs tests will be linked to the data base using only the unique identification number. No other identification data will be displayed.

In addition to the participants file, the ease-of-use questionnaire will include questions about: clarity of kit instructions, technical complexity, and ease of interpretation of results. See paragraph 4.10.

The data clerk and the study site investigator will monitor the quality of data collection and the study record keeping. Site investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Thereafter, data entry will be validated and any discrepancies verified against original data source documents and corrected.

Coded data will be exported from each site's source and merged into a single dataset for statistical analysis. A final analysis will be done considering pooled data in Epicentre, Paris.

A copy of the database will be sent and kept at Epicentre Paris. Data management and analyses will be performed using Stata 13® (StataCorp, College Station, Texas, USA).

## 5.2 Data analysis and study endpoints

Baseline characteristics of participants and samples will be described using summary statistics (mean or median and inter-quartile range for continuous variables, frequency and proportion for categorical variables). Distributions of categorical variables between classes will be compared using Chi-squared or Fisher's exact test. Comparisons of continuous variables between two groups will be performed with a t-test or a Wilcoxon rank sum test depending on their distributions. All estimates will be expressed with their respective 95% confidence interval, using exact confidence intervals as needed. The main analysis will be stratified by HIV status.

Following the algorithm detailed in the Fig.4, the HCV serology of each sample will be classified at the end as follow:

- positive: a positive EIA followed by positive RIBA result
- negative: a negative EIA results or a positive EIA followed by a negative RIBA result
- indeterminate: a positive EIA followed by an indeterminate RIBA

A separate description will be done for samples with an indeterminate result, but they will be excluded by the analysis.

Different analysis will be performed:

- Sensitivity and Specificity of each test performed by using a 2x2 contingency table.
- Percent agreement, misclassification and Kappa coefficient will be computed for the inter-reader comparison. A Kappa coefficient greater than 0.8 will be considered as a measure of good agreement.
- The distribution of the enrolled patients will be described according the following categories HCV VL (detectable and undetectable) and genotyping, HIV VL (1000 copies/ml), CD4 counts (CD4<250 and CD4>250 cells/ $\mu$ L) and HVB diagnosis (HBsAg reactive and non-reactive).
- Proportion of valid and invalid tests.
- Diagnostic odds ratio (DOR) to measure the effectiveness of the test.
- Positive and negative likelihood ratio (LR +/-).
- Positive and negative predictive values according to the source population prevalence.
- Ease-of-use.

The analysis will be completed with the description of the general characteristic of each test and the operational aspects of each RDT evaluated within this study.

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## **VII. ETHICAL CONSIDERATIONS**

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### **7.1 Ethical review**

Before the study is implemented, this study protocol will be submitted to the Ethics Review Board of MSF and ILBS. The study will be conducted in accordance with the principles of the Declaration of Helsinki (35) and international ethics guidelines for biomedical research on human subjects.

### **7.2 Informed consent**

The information sheet describing in detail the study procedures and risks will be given to the HIV cohort patient.

Consent form will be ERB-approved and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the patient and answer any questions that may arise. The patient will sign the informed consent document prior to any study-related assessments or procedures. The participant, if functionally illiterate, may sign the consent by marking a thumbprint on the signature line. They may withdraw consent at any time during the counseling and testing procedure. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the access to clinical care and the quality will not be affected if they decline to participate in this study.

Consent Form and Patient Information will be translated and back translated into the local languages most adapted to the patients (i.e Kouki and Apite for Churachandpur and Chakpikarong sites and Kouki and Burma in Moreh site).

### **7.3 Risks-benefits to participants**

#### **7.3.1 Risks to participants**

Study participation is not expected to convey any major risks to participants. The risk compared to normal procedures is associated with additional venous blood puncture where most current test protocols require only a finger prick. Venous blood collection is a very common procedure, which may cause minor pain and may cause slight discomfort.

Confidentiality will be ensured during the enrolment session and information collected will be used for study purposes only.

#### **7.3.2 Direct Benefits for Participants**

All study participants will benefit from the reference test (EIAs + RIBA) that represents an added value considering the limited data on the performance of RDTs in case of co-infection.

All the care will be provided free of charge. Results will be included in the patient file and the clinician will inform the patient in the next appointment date.

### **7.3.3 Indirect benefits for participants**

The study results are expected to support optimization of HCV screening in PLHIV. MSF operations will use the results to improve the accuracy of the testing procedures by changing testing devices or protocols. Access to the evaluated tests will depend of national registration. Furthermore, the information will be used to discuss with MoH and other testing facilities to improve accuracy based on the study outcome. Results will be also shared with different relevant authorities.

### **7.3.4 Risk protection**

Every effort will be made to minimize the risk of disclosure of the HIV and/or HCV status. Enrolment session will be conducted in a consultation room to ensure confidentiality during the consultation.

Only study staff and clinicians will have access to participants' study information. The stored samples and the database will be fully coded.

There will be no electronic link between the identification number and the name. Training of the staff will ensure to minimize any risk for the participants concerning the study.

Publications or scientific presentations of study findings will be presented using fully coded data.

## **7.4 Insurance of the study**

Epicentre, as the principal implementing agency will subscribe a study insurance to cover the participant's prejudices that may be directly linked with the participation in the study and with the procedures described in the study protocol.

## **7.5 Participant fee and incentives**

Participants in this study will not be compensated for their participation. Laboratory exams will be provided free of charge. The HIV and HCV treatment and the follow-up care are also free of charge if provided in the MSF facilities.

## **7.6 Confidentiality**

Patients' data will be confidential, coded and not shared with anyone outside of the study team unless this is necessary to protect the patient's health. The anonymity of the patient will always be ensured. The electronic database will not contain names or identification information of participants. Results generated by the study will not be released externally without the written permission of the participant, except as may be necessary for monitoring by regulatory authorities.

All study-related information will be stored securely at study sites. All participant information will be stored in locked file cabinets, in areas with access to staff only. All databases will be password-protected for security purposes. Forms, lists, logbooks and any other listings that link the participant identification numbers to other unique information will be stored in a separate, locked file in an area with limited access, only by the data manager and principal investigator or appropriate designee.

Participants' samples will be coded. The identification number will allow the link between the participants' information and sample/s. The EDTA tubes will be labeled with the identification number only. All study samples will be kept in a specific freezer on site and then in the referral laboratories. Access to these freezers and to the samples will be restricted to study staff only.

## **7.7 Dissemination of results and study findings**



Progress reports will be written and shared between the different partners: Epicentre, ILBS, Ethical Committees, MoH and co-investigators. .

**7.7.1 Community involvement and results dissemination to the community**

The community of PLHIV will be informed of the study and its rationale. This will be done through open meetings with the community and PLHIV specifically. Appropriate written materials in the local language will be made available. The results will be shared upon completion, through meetings with the community, PLHIV and local health professionals.

**7.7.2 Disseminating results to the key stakeholders and scientific community**

Results will be shared with the Health Authorities. Scientific papers will be prepared and submitted to peer-reviewed scientific journals. The results will also be presented at international conferences if relevant.

**VI. TIME FRAME**

The study inclusion period is expected to vary in different sites according to the national authorities’ approval.

Activities	Time
- Master Protocol development	- January 2017 - March 2017
- Submission to national Ethics Committees	- March 2017 - June 2017
- Field preparation and pilot study	- May 2017 - June 2017
- Recruitment of participants, sample collection, testing on site, preparation and storage	- July 2017 to December 2017
- Data analysis	- January 2018
- Final report (including outcomes)	- March 2018
- External communication	- June 2018
- Storage of remaining samples	- June 2017-April 2022

**Table 5:** Study time frame.

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## VIII. BIOHAZARD CONTAINMENT

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As the transmission of HCV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be taken by the personnel when drawing blood, handling of specimens and during testing. Transport and storage of the samples will be done following international recommendations to prevent any risk. The risk management will be adapted according to the different types of samples. In addition, all biological and chemical waste material will be disposed of accordingly.

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## IX. BUDGET

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The budget was estimated while taking into consideration the specificities of each site and the timeline of the activities. Some tests are part of the routine care so are not visible on this budget.

The estimated budget (Table 6) for all field study activities is expected to be as follows:

	US Dollars
Human Resources	15,000
Ethical submission and Insurance	10,000
Serological HCV RDTs	25,000
1 EIA + 1 RIBA	16,000
HBsAg	1,000
Transport and Storage	10,000
Office material	5,000
Travels	2,500
Meeting of the committees	2,500
Dissemination of results	3,000
Other miscellaneous costs	4,000
Total	94,000

**Table 6:** Estimated budget.

Epicentre will ensure the funding for this study with the UNITAID grant entitled “Ensuring access to the HCV treatment revolution for HCV/HIV co-infected patients in Low and Middle Income Countries”.

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## **APPENDICES**

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**Appendix A:** Patient Information Leaflet

**Appendix B:** Informed Consent Form

**Appendix C:** Workflow at each study site and storage of samples

**Appendix A: Patient Information about the study. *To be translated into the local language.***

**PATIENT INFORMATION LEAFLET**

Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients, *Study site, Year*

Madam / Sir,

You are being invited to take part in a study.

Before you decide whether or not to take part in the study, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information.

**1. What is the Hepatitis C virus (HCV) and testing?**

HCV is a liver disease caused by the hepatitis C virus. HCV can be a serious disease. The arrival of new and less toxic drugs means treatment access is evolving rapidly.

HCV screening relies on the use of rapid tests which detect if a person has been in contact with the virus. Afterwards, a confirmation test needs to be done to confirm if the infection is active. If a patient is confirmed with an active infection, additional tests will be performed before proposing a treatment if necessary. Today, screening and diagnosis are limited because of insufficient data on the disease in your country, because of the high cost of some tests and limited data on their performance. This study aims to evaluate different tests to push forward for a cheaper screening test in order to enlarge the access.

**2. What is the study about?**

This study aims to evaluate the performance of screening tests for HCV diagnosis. The study will compare the results obtained with the rapid tests with a reference technique.

This study is organized by Epicentre and the Institute of Liver and Biliary Science (ILBS) in New Delhi. This study is authorized by the authority of your country and an approval was granted from the competent ethical committees (name(s) of the ethical committee(s)) and the MSF ethical committee on the XX/XX/XXXX.

**3. Do I have to take part?**

It is up to you to decide whether or not to take part. You can also withdraw your consent at any time during the counseling and testing procedure without giving a reason. There will not be any financial expenses for you to participate in the study. You will not be paid to participate.

A decision to withdraw at any time during the counseling and testing procedure or a decision not to take part will not affect the standard of care you receive and your potential eligibility to HCV treatment.

**4. What will happen to me if I take part?**

If you accept to be part of the study, you will sign a consent form in two copies. One copy will be kept by us and one copy is for you. A study identification number will be assigned to you. Different demographic information will be collected on a form.

We will collect with your permission some blood: about two spoon's worth of blood, representing three vials of whole blood (12 ml). This blood collection will happen only once at the recruitment.

The samples will be used for the evaluation of the rapid tests. This evaluation will be partially performed in the laboratory of the health facility where you have been recruited and partially in the partner laboratory: ILBS (New Delhi). At ILBS, further analysis will be conducted using the reference standard (combination of two serological tests). This combination of tests ensures to get a more precise result about the presence or not of HCV antibodies in your blood. The presence of hepatitis B antibodies will be also tested. In addition, if the presence of HCV antibodies was confirmed in your blood the amount of hepatitis C virus (HCV viral load) and the type of the virus (genotype) will be assessed. HIV viral load to monitor the level of HIV virus will be also performed.

**5. What are the risks and advantages?**

Study participation is not expected to convey any major risks to you. The risk compared to the normal procedures is associated with additional venous blood. Venous blood is a blood drawn from a vein in your arm and it is a very common procedure which may cause minor pain and may cause slight discomfort.

You will receive the result of the standard test recognized by your health facility. In addition, you will benefit from the reference test performed at ILBS.

If the diagnosis of HCV chronic infection is confirmed, you will be managed normally in this health facility and if you are eligible you will be treated according to medical protocol. All the treatments and care will be provided free of charge.

The study results are expected to support better access and optimization of HCV patient screening. You and your community will indirectly benefit from the expected effects of the study findings on future HCV and follow-up cares.

#### **6. Will results be communicated?**

The results of the HCV screening test will be communicated to you.

In addition, your HIV VL and HBV status will be communicated and if the HCV reference test is positive you will receive the VL and the genotype. All these results will be discussed during one of your HIV consultation with your medical team, as your doctor.

#### **7. Privacy and confidentiality**

Confidentiality will be ensured during the enrolment session and information collected will be used for study purposes only.

The blood used for this study will not be labeled with your name so that your privacy will be guaranteed. Also, study records will contain only identification codes and will NOT show individual names.

#### **8. How will the collected data be treated?**

All information collected during the study will be confidential and coded. Your name will not be entered in an electronic database. Your medical record will be kept safe and only your medical and study teams can see it. We will not use your name in any study report. Every effort will be made to minimize the risk of disclosure of the HIV status or HCV status. There is no legal implication concerning the answers that you will give and the data that will be collected.

At completion of the study, a feedback in a form of a poster summarizing the main findings will be posted in the health center so that you will be informed of the results of the study. A scientific publication on the findings of the study is also planned in a peer-reviewed journal.

#### **9. Storage of specimen**

If you accept to give a blood sample, we will use part of it in the laboratory of this health facility and the remaining part will be sent to the reference laboratory (ILBS) in New Delhi.

If at the end of the above described laboratory procedures any sample will remain, then it will be stored in ILBS up to 5 years. We will store your samples with some information about you, such as your age and sex. No name will be written on the blood samples. There will be no way to know the blood sample is yours. It may be used for another purpose not directly linked to this study, with explicit authorization of the national authorities, who watch over the safety and rights of research participants. A research ethics board, which watches over the safety and rights of research participants, must approve any research study using your samples. Your sample will not be sold.

#### **10. What if there is a problem?**

If you have any questions, or if you are having any problem from any medicines, you should talk to the study nurse or doctor.

Name of a person to contact for questions:

(*Site investigator*) Tel: XXX XXX

Ethic committee: Tel XXXXXXX

The investigation team

**Appendix B: INFORMED CONSENT FORM. To be translated to the local language.**

**INFORMED CONSENT FORM**

Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients. *Study site, Year*

I, (first name, last name) \_\_\_\_\_ declare to have read, or have been explained by a study staff, the information notice concerning the study titled “Field evaluation of the performance of HCV Serological Rapid Diagnostic Tests among HCV/HIV co-infected patients” conducted by Epicentre and the Institute of Liver and Biliary Studies in New Delhi. I have clearly understood the objectives of the study. I have had the opportunity to ask questions about this research study. Any questions that I have asked have been answered to my satisfaction.

I have also understood that I can withdraw from the study at any time during the counseling and testing procedure without any prejudice or blame.

I agree to participate voluntarily in the study under the conditions presented in the information note.

**Participant:**

First name \_\_\_\_\_ Last name \_\_\_\_\_

Signature or Thumbprint \_\_\_\_\_ Place and date \_\_\_\_\_.

**Witness** (if patient is illiterate)

I, (first name, last name) \_\_\_\_\_ certify to all the necessary information on the study have been given and that the consent formed has been granted on a voluntary and free of constraints way.

Signature \_\_\_\_\_ Place and date \_\_\_\_\_

**Study clinician presenting the study:**

I, (position, first name, last name) \_\_\_\_\_ certify to have communicated to the participant all information on the study and its procedures. I pledge myself to comply with the terms of the protocol in the best way of confidentiality, respect of individual rights and liberty.

Signature \_\_\_\_\_ Place and date: \_\_\_\_\_

Persons to contact for further information regarding the study, the rights of participants, and in case of study related injury:

(Site investigator) Tel: XXX XXX

Ethic committee: Tel XXXXXXXX

2 COPIES: 1 FOR PATIENT, 1 FOR THE PATIENT’S CONFIDENTIAL RECORDS.



### Appendix C: Workflow at each study site and storage of samples

