

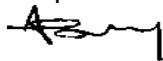
## Stratified Treatment OPTimisation for HCV-1 STOP-HCV-1

# Laboratory Manual For Imperial Processing and Storage

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**Written by:**  
**Name:** Aminata Sy  
**Role:** Trial Manager with Laboratory  
Responsibilities  
**Signature:** 

**Date:** 16-Oct-2017

**Name:** Graham Cooke  
**Role:** Chief Investigator  
**Signature:** 

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## 2 CONTACT INFORMATION

### 2.1 STOP HCV-1 CO-ORDINATING CENTRE- MRC AT UCL

<b>STOP HCV-1 Co-ordinating Centre - MRC CTU at UCL</b>		
MRC Clinical Trials Unit at UCL Institute of Clinical Trials & Methodology 90 High Holborn 2 <sup>nd</sup> Floor London WC1V 6LJ		Email: mrcctu.stophcv1@ucl.ac.uk Fax: 0207 670 4817
Clinical Operations Manager	Fleur Hudson	Email: mrcctu.stophcv1@ucl.ac.uk Tel: 0207 670 4782
Clinical Trial Manager	Emily Dennis	Email: mrcctu.stophcv1@ucl.ac.uk Tel : 0207 670 4660
Clinical Trial Manager	Cara Purvis	Email: mrcctu.stophcv1@ucl.ac.uk Tel: 020 7670 4930
Data Manager	Helen Ainscough	Email: mrcctu.stophcv1@ucl.ac.uk Tel: 020 7670 4652
Trial Manager with Laboratory Responsibilities	Aminata Sy	Email: a.sy@ucl.ac.uk Tel: 0207 670 4737

### 2.2 STOP HCV-1 IMPERIAL PROCESSING LABORATORY

<b>Imperial (Specimen Processing Laboratory)</b>	
Andrew Lovell/Maryam Khan Jefferiss Trust Labs Imperial College London Variety D Wing St Mary's Campus Norfolk Place, Paddington London W2 1PG	Email: a.lovell@imperial.ac.uk maryam.khan@imperial.ac.uk Tel: 02075943909

### 2.3 STOP HCV-1 CENTRAL ANALYTICAL LABORATORY

Olga Konopatskaya Peter Medawar Building for Pathogen Research South Parks Road Oxford OX1 3SY	Email: olga.konopatskaya@ndm.ox.ac.uk
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## 2.4 COURIER DETAILS FOR SHIPPING SPECIMENS TO IMPERIAL PROCESSING LABORATORY

Courier – City Sprint	
City Sprint	Tel: 02078801199
London Logistics	Email: UCL@citysprint.co.uk
1a Whitings Way	
London	Account number: CS027956
E6 6LR	
United Kingdom	

## 2.5 COURIER DETAILS FOR SHIPPING SAMPLES TO ANALYTICAL LABORATORY (OXFORD)

Courier	
PDP Courier Services Ltd	Tel: 01784 420466
Customer Services	Fax: 01784 424300
Apollo House	Email: customerservices@pdpcouriers.com
Plane Tree Crescent	
Feltham	<a href="#">*Please note MRC AT UCL will arrange the courier to the central lab</a>
Middlesex	
TW13 7HF	
United Kingdom	

### 3 SCOPE

This document provides information about procedures for collecting, processing, storing and shipping STOP HCV-1 trial samples. This document is for sites sending samples to Imperial for processing. (Please refer to Appendix III for a list of sites that will be following this Lab Manual).

This Lab Manual should only be followed if site is sending samples to Imperial for processing and storing.

## 4 SUMMARY OF TRIAL

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
<b>Acronym</b>	STOP-HCV-1
<b>Long Title of Trial</b>	Stratified Treatment Optimisation for HCV 1
<b>Version</b>	6.0
<b>Date</b>	17-Aug-2017
<b>ISRCTN #</b>	ISRCTN37915093
<b>EudraCT #</b>	2015-005004-28
<b>CTA #</b>	19174/0370/001-0001
<b>MREC #</b>	15/EE/0435
<b>Study Design</b>	An open-label randomised controlled trial (RCT) testing biomarker-stratified short-course first-line and re-treatment direct-acting antiviral (DAA) oral treatment regimens to cure mild chronic Hepatitis C (HCV) disease.
<b>Type of Patients to be Studied</b>	Adults ( $\geq 18$ years) infected with HCV genotype 1a/1b or 4 for $\geq 6$ months, with detectable plasma HCV RNA and mild liver disease (Fibroscan score F0-F1 or biopsy proven minimal fibrosis), HCV viral load $< 10$ million IU/ml, no previous DAA exposure (previous pegylated-interferon/ribavirin allowed) and not pregnant. Patients co-infected with HIV are eligible if HIV viral load has been $< 50$ copies/ml for $> 24$ weeks on anti-HIV drugs.
<b>Setting</b>	NHS
<b>Interventions to be Compared</b>	<p>The main intervention to be compared is varying (intervention) 4-7 weeks vs fixed (control) 8 weeks combination first-line DAA treatment, with or without ribavirin, in an open-label factorial design.</p> <ul style="list-style-type: none"> <li>Varying intervention duration will be stratified by baseline HCV RNA on a sliding scale, with duration determined by estimated time for HCV RNA to decline to reduce levels to <math>\sim 1</math> copy in the whole body at end of treatment.</li> <li>As soon as viral failure is detected at any time post-randomisation (first-line failure), patients will stop first-line treatment (if still receiving it) and be immediately retreated with 12 weeks of a different regimen.</li> <li>Ribavirin will be dosed twice daily, adjusted for weight</li> </ul> <p>Current first-line combination regimens are those licenced for use against Hepatitis C, namely:</p> <p>(i) a fixed dose combination of DAA active against genotype 1a/1b and 4; the Abbvie combination ombitasvir/paritaprevir/ritonavir (12.5mg/75mg/50mg) co-formulated film-coated tablets once daily (total daily dosage:</p>

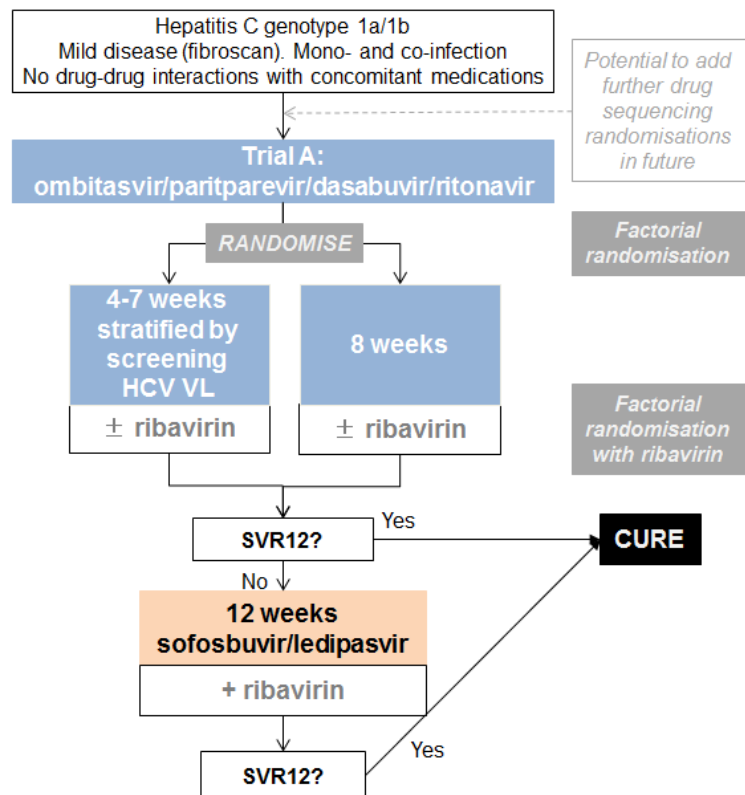
SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	<p>25/150/100mg) plus for genotype 1a/1b one dasabuvir 250 mg tablet twice daily (total daily dosage: 500mg) (using “ombitasvir/paritaprevir/(dasabuvir)/ritonavir” to denote the combination regimen)</p> <p>(ii) a fixed dose combination of 2 novel DAA active against all genotypes; the Abbvie combination glecaprevir/pibrentasvir (100mg/40mg) co-formulated tablets once daily (total daily dosage: 300/120mg)</p> <p>Current retreatment regimens are:</p> <p>(iii) a fixed dose double combination of sofosbuvir/ledipasvir (400mg/90mg) once a day plus ribavirin twice a day</p>
<b>Study Hypotheses</b>	<p>(i) HCV-RNA determined short-course (4-7 weeks) first-line will cure similar proportions with chronic, mild HCV disease as a fixed 8 week first-line course once failures have been retreated for 12 weeks</p> <p>(ii) Adjunctive ribavirin improves cure rates with biomarker-stratified short-course and fixed duration DAA first-line regimens</p> <p>(iii) Re-treatment with a longer 12 week regimen, given after detecting virological failure on or following first-line treatment, still achieve cures in the majority of the small proportion of patients failing first-line treatment.</p>
<b>Primary Outcome Measure</b>	<p>For the varying duration comparison the primary outcome will be:</p> <ul style="list-style-type: none"> <li>▪ Sustained Virological Response (SVR, plasma HCV RNA persistently &lt;LLOQ (lower limit of quantification)) measured 12 weeks after the end of the combined first and any re-treatment phases (SVR12)</li> </ul> <p>For the ribavirin comparison the primary outcome will be:</p> <ul style="list-style-type: none"> <li>▪ SVR12 after first-line treatment only</li> </ul>
<b>Secondary Outcome Measure(s)</b>	<ul style="list-style-type: none"> <li>▪ SVR12 after first-line treatment (where not the primary outcome)</li> <li>▪ SVR12 after the end of the combined first and any re-treatment phases (where not the primary outcome)</li> <li>▪ SVR24 after the end of the combined first and any re-treatment phases</li> <li>▪ SVR24 after first-line treatment only</li> <li>▪ lack of initial virological response</li> <li>▪ viral load rebound after becoming undetectable</li> <li>▪ serious adverse events</li> <li>▪ grade 3/4 adverse events</li> <li>▪ grade 3/4 adverse events judged definitely/probably related to interventions</li> <li>▪ treatment-modifying adverse events (any grade)</li> <li>▪ grade 3/4 anaemia</li> <li>▪ emergence of resistance-associated HCV variants</li> <li>▪ sensitivity/specificity of point-of-care diagnostic for IL28</li> <li>▪ costs and cost-effectiveness</li> </ul>
<b>Randomisation</b>	Patients will be allocated 1:1 using a factorial design to each of



SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	<ul style="list-style-type: none"> <li>• biomarker-stratified varying vs fixed duration</li> <li>• adjunctive ribavirin or not</li> </ul> <p>Randomisation will be stratified.</p>
<b>Number of Patients to be Studied</b>	408
<b>Duration</b>	<ul style="list-style-type: none"> <li>▪ Patients are planned to be recruited over 2 years</li> <li>▪ Each first-line intervention will be administered for 4-8 weeks</li> <li>▪ Each patient will be followed for 24 weeks post end of first-line treatment: if they fail first-line, they will receive another 12 weeks re-treatment and be followed for a further 24 weeks post end of re-treatment</li> <li>▪ The overall trial duration is planned for 4 years (including start-up and close-out)</li> </ul>
<b>Sponsor</b>	Imperial College London
<b>Funder</b>	Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership (14/02/17)
<b>Trial Manager</b>	Emily Dennis
<b>Chief Investigator</b>	Graham Cooke
<b>MRC CTU at UCL Project Leader</b>	Ann Sarah Walker

## 5 TRIAL SCHEMA

Figure 1:



**Follow-up:** day 3, 7, 14, 28, End of Treatment; then 4-weekly until 12 weeks post end of treatment, then at 24 weeks post end of treatment.

**Primary endpoint:** SVR12 (ie cure)

**Secondary endpoints:** SVR24; lack of initial virological response; viral load rebound (relapse) after becoming undetectable; serious adverse events, grade 3 or 4 adverse events, grade 3 or 4 adverse events judged definitely/probably related to the intervention; treatment-modifying adverse events of any grade, grade 3 or 4 anaemia; emergence of resistance-associated Hepatitis C variants

## 6 TRIAL ASSESSMENT SCHEDULE

**Table 1 Trial Assessment Schedule – first-line treatment real-time tests (see Table 2 for first-line sample storage)**

	SCREENING <sup>†</sup>	DAY POST RANDOMISATION*					EOT	WEEK POST EOT			
		0	3	7	14	28		4	8	12	24
Control: 8 weeks treatment [continuing]		DAA	[DAA]	[DAA]	[DAA]	DAA		<i>(see retreatment schedule below for any treatment after first-line EOT)</i>			
Intervention maximum: 7 weeks treatment [continuing]		DAA	[DAA]	[DAA]	[DAA]	DAA					
Intervention minimum: 4 weeks treatment [continuing]		DAA	[DAA]	[DAA]	[DAA]	DAA					
Eligibility assessment	X										
Patient information sheet and consent	X										
Randomisation		X									
Clinical assessment <sup>(a)</sup>		X	X	X	X	X	X	X	X	X	X
Self-reported adherence			X	X	X	X	X				
Fibroscan or biopsy**	(X)										
Weight (kg)	X					X	X	X		X	X
Height (m)	X										
Urine pregnancy test if child-bearing potential		X				X	X			X	X
Quality of life <sup>(b)</sup>		X					X			X	
EDTA blood for haematology <sup>(c,h,i)</sup> (5ml)	(X)	X			X	X	X			X	X
Clotted blood for biochemistry <sup>(d,h,i)</sup> (5ml)	(X)	X			X	X	X			X	X
Coagulation markers (2.5ml)	(X)										
Real-time HCV viral load <sup>(h,i)</sup> (10ml)	(X)	X	X	X	X	X	X	X	X	X	X
Point of care IL28 polymorphism test (Epistem) <sup>(e,g)</sup>		X									
Total blood draw in ml for real-time tests	-	20	10	10	20	22.5	22.5	10	10	22.5	22.5
<b>If HIV-infected</b>	(X)						X				X
- HIV viral load (9ml) (additional)	(X)						(X)				(X)
- CD4 cell count <sup>(f)</sup>											

(j) indicate tests that will have already been performed as part of standard management, but results will be recorded for the trial. Screening blood tests should have been performed within 60 days prior to randomisation.

On treatment visits should be within  $\pm 1$  day of the nominal visit day and end of treatment (EOT) visits within  $\pm 3$  days of the nominal visit day. The Day 3 visit must occur 3 or more calendar days before the Day 7 visit (that is, there should be two calendar days completely separating them). Any patient with a single HCV RNA >lower level of quantification (LLOQ) after two consecutive HCV RNA <LLOQ, or with a single value >2000 IU/ml and >1 log<sub>10</sub> increase above the HCV RNA nadir on treatment or post EOT should be recalled for a second HCV RNA test at least one week after the initial value to confirm whether or not failure has occurred. Quality of life should also be assessed at this confirmation of failure visit.

\* If a patient fails at any time point from day 14, then they move to the flow sheet for re-treatment below.

† Screening visit may be any time up to 60 days prior to randomisation, since patients with mild disease will be stable.

\*\* Fibroscan or biopsy may be conducted within 180 days of randomisation

- (a) Including record of concomitant medications, grade 3 or 4 or serious adverse events, adverse events (including reactions) of any grade leading to treatment modification including interruption/early discontinuation, resource utilisation, pill count.
- (b) Quality of life will be assessed using the EuroQol (5 dimensions) (EQ-5D), the Medical Outcomes Study Short-Form 12 Item Survey<sup>1</sup> (SF-12, version 2) and the Cognitive Function Scale<sup>2</sup> (MOSCOG). Quality of life should also be performed at any additional visits to confirm HCV viral load failure.
- (c) For real-time measurement of haemoglobin, white cell count, lymphocytes, neutrophils, platelets.
- (d) For real-time measurement of alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, albumin and creatinine, and calculation of creatinine clearance (Cockcroft Gault).
- (e) Only with specific consent for genetic testing.
- (f) Screening CD4 cell count from within 1 year of randomisation can be used.
- (g) EPISTEM test can be done at any time point if not possible on day 0.
- (h) If a participant is hard to bleed, the blood tests should be prioritised as follows: Biochemistry>haematology (FBC>differential>)>HCV viral load> storage.
- (i) If unable to bleed on day 28, EOT or post-EOT week 12, the patient should be recalled, as these are critical visits for clinical care.

**Table 2 Sample Collection Schedule – first-line treatment sample storage**

	DAY POST RANDOMISATION*					EOT	WEEK POST EOT			
	0	3	7	14	28		4	8	12	24
Storage: sites processing all samples locally										
EDTA plasma for local storage (20ml blood)	X					X			X	
EDTA plasma for local storage (10ml blood)		X	X	X	X		X	X		X
EDTA whole blood for local DNA storage <sup>(a,b)</sup> (2.5ml)	X									
Whole blood in PAXgene blood RNA tube (Qiagen) <sup>(a)</sup> (2.5ml)	X									
Storage: PBMC (20ml) <sup>(c,d)</sup>	X	X	X			X	X			
Total storage sample blood draw in ml	45	30	30	10	10	40	30	10	20	10

(a) Only with specific consent for genetic testing.

(b) Can be taken at any time point if not possible on day 0.

(c) Only in a subset of sites with capacity to extract cells.

(d) If day 0 taken, up to a maximum of 4 other timepoints will be collected with EOT being most important.

The collection on day 0 can be taken at either screening or day 0.

Samples may be shipped outside of the UK to North America or Europe for additional tests after the end of the trial

**Table 3 Trial Assessments and Sample Collection Schedule – Re-treatment**

	START OF RE-TREATMENT (0) *	WEEKS FROM START OF RE-TREATMENT							
		2	4	8	12 (EOT)	16 EOT+4	20 EOT+8	24 EOT+12	36 EOT+24
12 weeks treatment [continuing]	DAA	[DAA]	DAA	DAA					
Clinical assessment <sup>(a)</sup>	X	X	X	X	X	X	X	X	X
Self-reported adherence		X	X	X	X				
Weight (Kg)	X		X	X	X	X		X	X
Urine pregnancy test if child-bearing potential	X		X		X			X	X
Quality of life <sup>(b)</sup>	X				X			X	
EDTA blood for haematology <sup>(c,g,h)</sup> (5ml)	(X)	X	X	X	X			X	X
Clotted blood for biochemistry <sup>(d,g,h)</sup> (5ml)	(X)	X	X	X	X			X	X
Coagulation markers <sup>(g)</sup> (2.5ml)	(X)		X						
Real-time HCV viral load <sup>(g,h)</sup> (10ml)	(X)	X	X	X	X	X	X	X	X
Storage: sites processing all samples locally									
EDTA plasma for storage <sup>(g,h)</sup> (10ml blood)	(X)	X	X	X	X	X	X	X	X
Total blood draw in ml if storing locally	32.5	30	32.5	30	32.5	20	20	32.5	32.5
<b>If HIV-infected</b>	X				X				X
- HIV viral load (9ml) (additional)	(X)				(X)				(X)
- CD4 cell count									

\* If laboratory tests and plasma storage have already been performed in the prior 7 days as part of the first-line schedule above, then they do not need to be repeated at the start of re-treatment.

(a) Including record of concomitant medications, grade 3 or 4 or serious adverse events, adverse events of any grade leading to treatment modification including interruption/early discontinuation, resource utilisation, pill count;

(b) Quality of life will be assessed using the EuroQol (5 dimensions) (EQ-5D), the Medical Outcomes Study Short-Form 12 Item Survey<sup>1</sup> (SF-12, version 2) and the Cognitive Function Scale<sup>2</sup> (MOSCOG).

(c) For real-time measurement of haemoglobin, white cell count, lymphocytes, neutrophils, platelets

- (d) For real-time measurement of alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, albumin and creatinine, and calculation of creatinine clearance (Cockcroft Gault).
- (g) If a participant is hard to bleed, the blood tests should be prioritised as follows: Biochemistry>haematology (FBC>differential>INR)>HCV viral load> storage.
- (h) If unable to bleed on week 4, EOT or post-EOT week 12, the patient should be recalled, as these are critical visits for clinical care.

## 7 LABORATORY FACILITY REQUIREMENTS

The laboratories used in the STOP-HCV-1 trial to locally process and store trial specific specimens must meet the following requirements listed below:

- Be able to process all appropriate specimens within the timeframe stipulated in this manual
- Have appropriate equipment, such as centrifuges that can reach a minimum speed of 2000 g
- Have appropriate storage space available in a -70/-80°C freezer
- A freezer alarm system is required to alert staff if a freezer fails and a plan needs to be in place to ensure that the specimens remain at the appropriate temperature.
- A local standard operating procedure must be in place to respond to incidents that threaten the integrity of the STOP-HCV-1 samples – incidents such as power failures, freezer failures, storms etc. The STOP-HCV-1 team at MRC CTU should be informed of any incident of temperature deviation.
- Under no circumstances must personal information (e.g. participant name, initials, doctor name etc.) be put on any vial, container, label or form that may be seen outside the local processing area.



## 8 MATERIALS PROVIDED

### 8.1 MATERIALS TO BE PROVIDED BY CLINICS FOR USE IN STOP HCV-1 TRIAL SPECIMEN COLLECTION

- Blood Collection Containers: EDTA (Lavender top)

### 8.2 MATERIALS TO BE PROVIDED BY STOP HCV-1 TRIAL TEAM TO CLINICS

- Pre-printed Thermal Transfer Labels: For use on blood collection tubes
- Forms: STOP HCV-1 Lab Request forms

### 8.3 MATERIALS TO BE PROVIDED BY STOP HCV-1 TRIAL TEAM TO IMPERIAL PROCESSING LABORATORY

- Cryovials: For storage of Plasma and Whole Blood (for DNA extraction) aliquots (Sarstedt, catalogue no. 72.694.005)
- Cryoboxes and cryobox inserts: For storage of Plasma aliquots and Whole Blood (for DNA extraction) Fisherbrand 81-place (9 x 9) (Supplier: Fisher Scientific Cat. Nos: FB71211 and FB71305 respectively)
- PAXgene RNA blood tubes (BD Biosciences, Cat No: 762165)
- Cryoboxes and cryobox inserts for PAXgene RNA tubes: Fisherband 49 place (7x7) (Supplier: Fisher Scientific FB71247 and FB71333 respectively)
- Pre-printed Thermal Transfer Labels: For use on 2.0mL cryovials
- Forms: Specimen Storage and Shipping Logs, laboratory signature logs

### 8.4 MATERIALS TO BE PROVIDED BY COURIER FOR SHIPMENT FROM STOP HCV-1 PROCESSING TO ANALYTICAL LABORATORY

The courier will provide adequate packaging for UN 3373 Biological Substance, Category B and UN1845 Dry Ice.

## 9 SPECIMEN LABELS

### 9.1 LABELLING BLOOD COLLECTION TUBES (CLINICS)

For use on the EDTA blood collection tubes or PAXgene collection tubes when collecting specimens as specified in the collection table (**Section 11**). Specimens must be labelled at clinic by person collecting bloods from participants.

<p><b>TRIAL NAME = STOP HCV-1</b></p> <p><b>PARTICIPANT TRIAL ID= PID</b> Trial code = H Site number = Three digits after letter H e.g. 113 Participant Number= _ _ _ _</p> <p><b>COLLECTION =</b> labels for clinics</p> <p><b>VISIT=</b> Study visits e.g. FL-DAY 0</p> <ul style="list-style-type: none"> <li>• FL- denote first-line treatment</li> <li>• R- denotes Re-treatment</li> </ul> <p><b>DATE =</b>Date of sample collection</p>	<p><b>A</b></p> <p>STOP HCV-1 <small>Collection 1</small> PID: H113 _ _ _ _ Visit: FL-Day 0 PLASMA S00584631 Date _ / _ / _</p> <p><b>B</b></p> <p>STOP HCV-1 <small>Collection 1</small> PID: H113 _ _ _ _ Visit: FL-Day 0 WHOLE BLOOD S00584644 Date _ / _ / _</p> <p><b>C</b></p> <p>STOP HCV-1 <small>PAXgene</small> PID: H113 _ _ _ _ Visit: FL-Day 0 WHOLE BLOOD S00584648 Date _ / _ / _</p>
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**THE FOLLOWING FIELD MUST BE  
HANDWRITTEN ON LABELS**

- **DATE**
- **PID 3 DIGITS AND LETTER**

**Figure 1: Understanding specimen collection labels**

- Write all missing fields on the label using **permanent ink**
- Ensure the labels are attached properly to the Blood Collection Tubes
- Ensure all collected specimens are labelled before sending to the lab
- If you need additional labels for any of the specimens please contact the STOP HCV-1 team: [mrcctu.stophcv1@ucl.ac.uk](mailto:mrcctu.stophcv1@ucl.ac.uk)
- The Laboratory Request Form (**Appendix II**) should accompany the specimens to the lab (duplicate this forms if necessary).

## 9.2 LABELLING CRYOVIALS (IMPERIAL PROCESSING LABORATORY)

Blood specimens will be transported from the clinic to the processing lab and stored following processing. Laboratory technicians/personnel must ensure all samples are labelled prior to storage.

**TRIAL NAME = STOP HCV-1**

**PARTICIPANT TRIAL ID= PID**  
 Trial code = H  
 Site number = Three digits after letter H e.g. 113  
 Participant Number= \_\_\_\_\_

**COLLECTION =** labels for clinics

**VISIT=** Study visits e.g. FL-DAY 0

- FL- denote first-line treatment
- R- denotes Re-treatment

**DATE =**Date of sample collection

**A**

Aliquot 1

STOP HCV-1  
PID: H113\_ \_ \_ \_  
Visit: FL-Day 0 PLASMA  
S00584635 Date \_ / \_ / \_

**B**

STOP HCV-1 Aliquot 1  
PID: H113\_ \_ \_ \_  
Visit: FL-Day 0 WHOLE BLOOD  
S00584645 Date \_ / \_ / \_

**C**

STOP HCV-1 PAXgene  
PID: H113\_ \_ \_ \_  
Visit: FL-Day 0 WHOLE BLOOD  
S00584648 Date \_ / \_ / \_

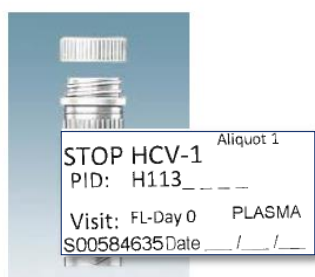
**THE FOLLOWING FIELD MUST BE  
HANDWRITTEN ON LABELS**

- **DATE**
- **PID 3 DIGITS AND LETTER**

**Figure 2: Understanding sample processing labels**

- Ensure the vial is dry and not frozen before attaching the label
- Attach label to the vial horizontally **leaving one centimetre between the label and the bottom of the vial**
- Stick the left edge of the label to the cryovial first to prevent overlap which could obscure the label
- Do not write any additional information on the label other than the items listed above.

Please attach label as shown in the diagram below.



## 10 SAMPLE STORAGE AND TRACKING

### 10.1 SAMPLE STORAGE

Samples will be stored in different grid boxes following processing depending on sample type, Whole Blood, PAXgene tubes and Plasma will be stored in separate cryoboxes.

In addition 2 plasma aliquots from FL-Day-0 visit will be stored in an additional separate box.

Every grid box must be unique and will be identified with a Grid Box Label by STOP HCV-1 Team as follows:

- 7X7 cryobox labelled IMPAX001 = PAXgene tubes stored at -80°C
- 9x9 cryobox labelled IMBL001 = Whole Blood stored at -80°C.
- 9x9 cryobox labelled IMPLA001 = Plasma all time points stored at -80°C
- 9x9 cryobox labelled IMFL001 = FL-Day 0 2x Plasma aliquot stored at -80°C

### 10.2 SAMPLE TRACKING

**Note:** Please ensure to start with Box #1 when using the boxes provided, these supplied boxes have the Box Number written on them already.

Each 9x9 cryobox has 81 aliquots positions, each position is numbered and it is these numbers which are used to indicate the location of each aliquot on the lab request form and in the sample inventory log (**Appendix I**).

Care must be taken to ensure samples are placed in the box starting at position 1 and moving sequentially through to position 81. Store aliquots as they are received, do not group all samples for all visits for one participant together. Do not leave empty spaces in the box. See **Figure 3** below which details how to store samples.

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	Etc.				

**Figure 4: Placing aliquots in the storage box in the correct order**

### 10.3 COMPLETEING THE STOP HCV-1 SPECIMEN LOG

- Specimens should be logged on the STOP-HCV-1 electronic specimen log (Appendix I).
- Each ample type has a separate tab on the log – Plasma FL Day 0, Plasma all time points, Whole blood and PAXgene.
- Please ensure to complete the columns on the logs as follows:
- Site – full name or number
- PID – participant number
- Visit – use the same format as in table 1 and table 2, for example FL-Day 28, R-Week 0.
- Date of Collection – date sample collected
- Sample type – this will be pre-filled, for example Plasma.
- Box number – This will be on the label attached to each cryobox
- Position in box – start in the top left hand corner and count from left to right 1-9, then move to the left of the 2<sup>nd</sup> row down and count from 10-19, and so on.
- Date shipped – complete on day of shipping.

## 11 STOP HCV-1 SPECIMENS: COLLECTION TABLES

**Important: This table does not include the blood needed for routine bloods taken as part of the STOP HCV-1 trial.**

**TABLE 1: FISRT LINE (FL) TREATMENT SPECIMEN COLLECTION**

Visit	Specimen	Type of Collection Container	Volume of Specimen Collected	Stored as	Type of storage container
<b>FL-Day 0</b>	Plasma	EDTA Blood Collection Tube	<b><u>20 ml</u></b>	6 x 1 ml	Cryovial
	Whole Blood (DNA Isolation)	EDTA Blood Collection Tube	2.5 ml	2 x 1.25 ml	Cryovial
	Whole Blood (RNA Isolation)	PAXgene tube	2.5ml	1 x 2.5 ml	PAXgene tube
<b>FL-Day 3</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-Day 7</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-Day 14</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-Day 28</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-EOT</b>	Plasma	EDTA Blood Collection Tube	<b><u>20 ml</u></b>	6 x 1 ml	Cryovial
<b>FL-EOT+4</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-EOT+8</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>FL-EOT+12</b>	Plasma	EDTA Blood Collection Tube	<b><u>20 ml</u></b>	6 x 1 ml	Cryovial
<b>FL-EOT+24</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial

FL = First Line  
EOT = End of Treatment

Please note standard Plasma draw is 10 ml except at **FL-Day 0, FL-EOT, FL-EOT+12** when it is **20 ml**.

- 1 All participants will have specimens collected for storage on FL-Day 0, FL-Day 3, FL-Day 7, FL-Day 14, FL-EOT, FL-EOT+4, FL-EOT+8, FL-EOT+12 and FL-EOT+24.
- 2 In the control group (randomised to 56 days of treatment) participants will also have specimens collected for storage on FL-Day 28 (their 56 day sampling is defined as FL-EOT)
- 3 Participants randomised to varying durations (between 28-49 days) will have specimens collected depending on the number of days.
- 4 If the treatment duration falls on exactly FL-Day 28 then this is defined as FL-EOT

**TABLE 2: RE-TREATMENT (R-) SPECIMEN COLLECTION**

Visit	Specimen	Type of Collection Container	Volume of Specimen Collected	Stored as	Type of storage container
<b>R-Week 0*</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-Week 2</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-Week 4</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-Week 8</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-EOT</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-EOT+4</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-EOT+8</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-EOT+12</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
<b>R-EOT+24</b>	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial

\*If laboratory tests and plasma storage have already been performed in the prior 7 days as part of the first line schedule above, they do not need to be repeated at the start of re-treatment.

## 12 STOP HCV-1 COLLECTION PROCEDURES

### 12.1 COLLECTION OF BLOOD FOR PLASMA PROCESSING

Refer to **Section 11** to determine collection requirements at a given visit for processing.

- 1) Collect the participant's blood in **EDTA blood collection tubes**
- 2) Gently invert the tube 8-10 times to mix the blood and additive
- 3) Maintain blood at room temperature and centrifuge within 4 hours of collection
- 4) Centrifuge the blood at room temperature at 2000g x 20 minutes so the plasma and cells are separated
- 5) Before aliquoting pre-label the 2.0 ml Sarstedt tubes with STOP HCV-1 labels
- 6) Using a graduated transfer pipette and following aseptic techniques remove the plasma layer without disturbing the red layer. Transfer 1 ml plasma to each of the cryovial
- 7) Immediately freeze for storage in 81 grid box and document the position on the STOP HCV-1 Specimen log (See **Appendix I**)\*

\* Please note at visit FL-Day 0 participants have 2 x aliquots stored in a separate grid box (and this is documented on a separate Specimen Log)

### 12.2 COLLECTION OF BLOOD FOR WHOLE BLOOD (DNA ISOLATION) PROCESSING

- 1) Collect the participant's blood in **EDTA BLOOD COLLECTION TUBE**
- 2) Gently invert the tube 8-10 times to mix the blood and additive **DO NOT CENTRIFUGE**
- 3) Before aliquoting pre-label the 2.0 ml Sarstedt tubes with STOP HCV-1 labels
- 4) Using a graduated transfer pipette and following aseptic techniques transfer half of the sample into to each of the cryovials (there should be ~1.25 ml in each tube)
- 5) Immediately freeze for storage in 81 grid box and document the position on the STOP HCV-1 Specimen log (See **Appendix I**)

### 12.3 COLLECTION OF BLOOD FOR WHOLE BLOOD (RNA ISOLATION) PROCESSING

- 1) Collect the participant's blood in a **PAXgene RNA tube** (BD Biosciences); ensure this is the **last tube drawn** in phlebotomy procedure.
- 2) Maintain tube upright at room temperature (18°C to 25°C) for a minimum of 2hr and a maximum of 72hr before transferring to a freezer (-20°C)
- 3) Freeze for 24hr at -20°C then transfer to -70/80°C for storage as recommended by the manufacturer
- 4) Complete the PAXgene specimen log (**Appendix I**)



## 13 STOP HCV-1 SPECIMEN SHIPPING PROCEDURES

### 13.1 SHIPPING STOP HCV-1 SPECIMENS FROM CLINCS TO PROCESSING LABORATORY

Please note blood samples need to be collected and couriered as soon as possible.  
Having labelled all the specimens and completed the laboratory request form:

- ✓ Place in PathoPouch and PathoShield box with Lab Request form (duplicate this form if necessary)
- ✓ Arrange courier collection for delivery to STOP HCV-1 Specimens to Processing Laboratory (as described below):
  - E-mail or call Courier (City Sprint) refer to (**Section 2.4**) for contact information  
Please remember to quote the following when making the booking:
  - Collection address of specimens
  - Account number: **CS027956**
  - Destination address of specimens (listed in **Section 2.2**)
  - Ambient temperature, same day standard delivery

Ensure you package the blood tubes in patho pouch from your site and that samples are collected **by 12:00pm**

### 13.2 SHIPPING STOP HCV-1 SAMPLES FROM PROCSSING LABORATORY TO ANALYTICAL LABORAORY (OXFROD)

Samples processed at Imperial will remain at the laboratory until STOP HCV-1 team contact to centralise the samples in Oxford.

Prior to shipment please ensure that:

- The specimens are correctly labelled and the position of the specimen in the box matches the position of the label on the log.
- The specimen logs are faxed/emailed to the STOP HCV-1 Coordinating Centre before the shipment date.

On the day of the shipment:

- Please make sure all specimens and paperwork are completed correctly
- Place the original Specimen Log (**Appendix I**) in the package and keep a copy for your records.
- Complete and sign all the documents provided by the courier.
- The person responsible for the shipment at site must be able to be contacted before and during each shipment.

In case of query, the CTU will contact the Site/Laboratory in order to resolve them and to improve procedures (when applicable) for the next shipment.

## APPENDIX I SPECIMEN LOGS



### Plasma- All Timepoints Specimen Log/Inventory

Site Name:

Site Number:  
Location of Samples:  
Storage temperature : -80

Site	PID	Visit	Date of Collection	Sample Type	Box Number	Position in Box	Date shipped
				Plasma	1	1	
				Plasma	1	2	
				Plasma	1	3	
				Plasma	1	4	
				Plasma	1	5	
				Plasma	1	6	
				Plasma	1	7	
				Plasma	1	8	
				Plasma	1	9	
				Plasma	1	10	
				Plasma	1	11	
				Plasma	1	12	
				Plasma	1	13	
				Plasma	1	14	
				Plasma	1	15	



### Plasma- FL DAY 0 Specimen Log/Inventory

Site Name:

Site Number: 91  
Location of Samples:  
Storage temperature : -80

Site	PID	Visit	Date of Collection	Sample Type	Box Number	Position in Box	Date shipped
				Plasma	1	1	
				Plasma	1	2	
				Plasma	1	3	
				Plasma	1	4	
				Plasma	1	5	
				Plasma	1	6	
				Plasma	1	7	
				Plasma	1	8	
				Plasma	1	9	
				Plasma	1	10	
				Plasma	1	11	
				Plasma	1	12	
				Plasma	1	13	
				Plasma	1	14	



### PAXgene- Specimen Log/Inventory

Site Name:

Site Number:  
Location of Samples:  
Storage temperature : -80

Site	PID	Visit	Date of Collection	Sample Type	Box Number	Position in Box	Date shipped
				PAX gene	1	1	
				PAX gene	1	2	
				PAX gene	1	3	
				PAX gene	1	4	
				PAX gene	1	5	
				PAX gene	1	6	
				PAX gene	1	7	
				PAX gene	1	8	
				PAX gene	1	9	
				PAX gene	1	10	
				PAX gene	1	11	
				PAX gene	1	12	
				PAX gene	1	13	
				PAX gene	1	14	
				PAX gene	1	15	




### Whole Blood (DNA Isolation)- Specimen Log/Inventory

Site Name:

Site Number:  
Location of Samples:  
Storage temperature : -80

Site	PID	Visit	Date of Collection	Sample Type	Box Number	Position in Box	Date shipped
				Whole Blood	1	1	
				Whole Blood	1	2	
				Whole Blood	1	3	
				Whole Blood	1	4	
				Whole Blood	1	5	
				Whole Blood	1	6	
				Whole Blood	1	7	
				Whole Blood	1	8	
				Whole Blood	1	9	
				Whole Blood	1	10	
				Whole Blood	1	11	
				Whole Blood	1	12	
				Whole Blood	1	13	
				Whole Blood	1	14	
				Whole Blood	1	15	

**APPENDIX II STOP HCV-1 LAB REQUEST FORM**

	Attach Participant ID label	<b>Laboratory Request Form</b> <b>Version 1.0 16-Mar-2016</b>
		Visit Date: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
		First line      Day <input type="text" value=""/> <input type="text" value=""/> EOT <input checked="" type="checkbox"/> Retreatment    Week <input type="text" value=""/> <input type="text" value=""/> EOT <input checked="" type="checkbox"/>

Complete this form when collecting STOP HCV-1 stored samples.  
 Send original with specimens to STOP HCV-1 Processing Lab and file a copy at site

<b>A. PLASMA</b>	Date Specimen obtained: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
20 ml Required at First Line Day 0, EOT and EOT+12 10 ml Required at First line: day 3, 7, 14, 28, 42, FL-EOT+4, FL-EOT+8, FL-EOT+24 Retreatment: week 0, 2, 4, 8, 12(EOT), R-EOT+4, R-EOT+8, R-EOT+12, R-EOT+24	
Collected in EDTA tubes	Stored
Size of collection tubes	Number of tubes collected
<input type="text" value=""/> <input type="text" value=""/> • <input type="text" value=""/> ml	<input type="text" value=""/> <input type="text" value=""/>
	Number of aliquots stored
	<input type="text" value=""/> <input type="text" value=""/>
<input type="checkbox"/> Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels	

<b>B. WHOLE BLOOD (DNA ISOLATION)</b>	Date Specimen obtained: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
2.5 ml Required at First Line Day 0	
Collected in EDTA tubes	Stored
Size of collection tubes	Number of tubes collected
<input type="text" value=""/> <input type="text" value=""/> • <input type="text" value=""/> ml	<input type="text" value=""/> <input type="text" value=""/>
	Number of aliquots stored
	<input type="text" value=""/> <input type="text" value=""/>
<input type="checkbox"/> Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels	

<b>B. WHOLE BLOOD (RNA ISOLATION)</b>	Date Specimen obtained: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
2.5 ml Required at First Line Day 0	
Collected in PAXgene tubes	Stored
Size of collection tubes	Number of tubes collected
<input type="text" value=""/> <input type="text" value=""/> • <input type="text" value=""/> ml	<input type="text" value=""/> <input type="text" value=""/>
	Number of PAXgene tubes stored
	<input type="text" value=""/> <input type="text" value=""/>
<input type="checkbox"/> Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels	

**Samples sent by:**

Signature: <input style="width: 95%;" type="text"/>	Printed Name: <input style="width: 95%;" type="text"/>	Date Completed: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
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**Samples processed by:**

Signature: <input style="width: 95%;" type="text"/>	Printed Name: <input style="width: 95%;" type="text"/>	Date Completed: <input type="text" value="d"/> <input type="text" value="d"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="m"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/> <input type="text" value="y"/>
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Place aliquots into corresponding grid box and document on Specimen Log with STOP HCV-1 Specimen Log Label.

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## APPENDIX III: SITES SENDING SPECIMENS TO IMPERIAL FOR PROCESSING

- 8) 091- Royal Free Hospital
- 9) 349- Chelsea and Westminster Hospital