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STOP-HCV Stratified Medicine for HepC

Stratified Treatment OPtimisation for HCV-1
STOP-HCV-1

Laboratory Manual For Local Processing and Storage

Version: 4.0
Date: 01-Nov-2017

ISRCTN #: ISRCTN#37915093

EUDRACT #: 2015-005004-28
CTA #: 19174/0370/001-0001
MREC#: 15/EE/0435

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Version	Date	Author	Reason for revision
1.0	16-Mar-2016	Charlotte Russell	Initial
2.0	30-Jan-2017	Charlotte Russell	Release of protocol v4.0
3.0	30-Jun-2017	Emily Dennis	Release of protocol v5.0
4.0	01-Nov-2017	Emily Dennis	Release of protocol v6.0

1 CONTENTS

2 CONTACT INFORMATION

2.1 STOP-HCV-1 CO-ORDINATING CENTRE – MRC CTU AT UCL

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2.2 STOP-HCV-1 SPECIMEN RECEIVING LABORATORY

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2.3 COURIER FOR SHIPPING SPECIMENS FROM STOP HCV-1 CLINICS TO STOP HCV-1 SPECIMEN RECEIVING LABORATORY

Courier	
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3 SCOPE

This document provides information about site procedures for collecting, processing, storing and shipping specimens for the STOP-HCV-1 trial for the sites are processing locally (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 the site is processing and storing the samples collected on site. For sites that are part of HCV Research UK and will send specimens to Glasgow via DX boxes, or those sending samples to Imperial, they should refer to those specific instructions not contained in this manual.

4 SUMMARY OF TRIAL

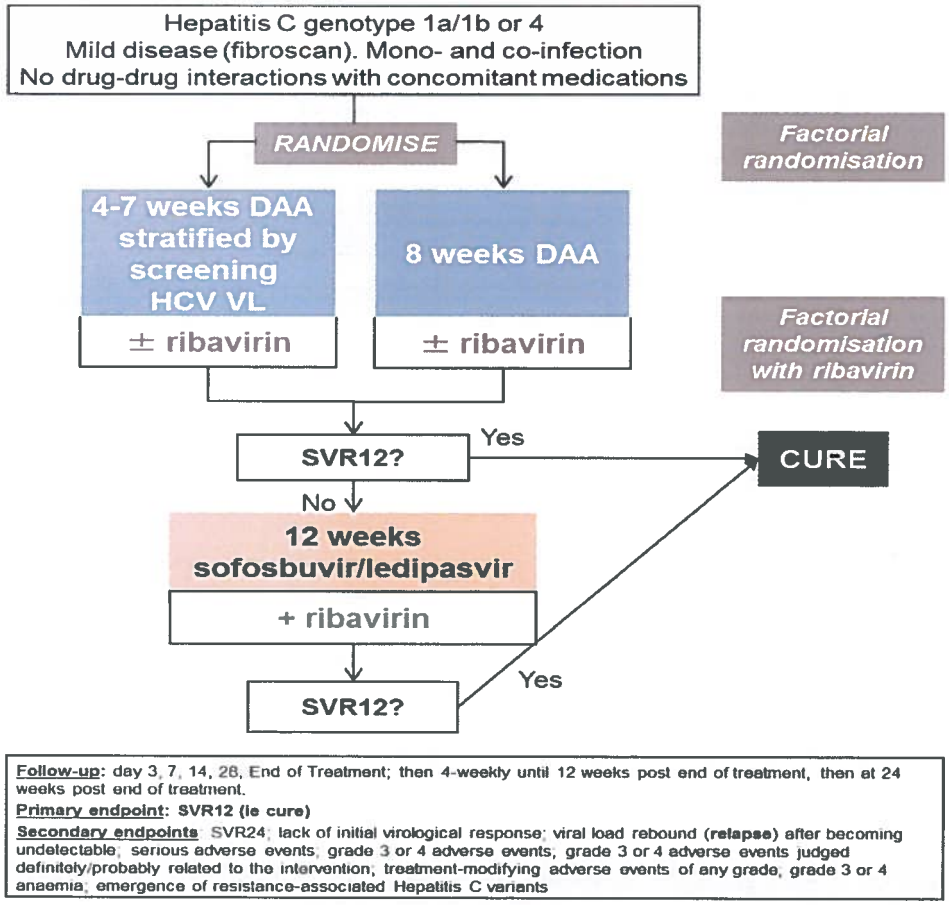
SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Acronym	STOP-HCV-1
Long Title of Trial	**Error! Reference source not found.**
Version	6.0
Date	17-Aug-2017
ISRCTN #	ISRCTN37915093
EudraCT #	2015-005004-28
CTA #	19174/0370/001-0001
MREC #	15/EE/0435
Study Design	An open-label randomised controlled trial (RCT) 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.
Type of Patients to be Studied	Adults (≥18 years) infected with HCV genotype 1a/1b or 4 for ≥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.
Setting	NHS
Interventions to be Compared	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. • 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 ~1 copy in the whole body at end of treatment. • 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. • Ribavirin will be dosed twice daily, adjusted for weight Current first-line combination regimens are those licenced for use against Hepatitis C, namely: (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-

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	formulated film-coated tablets once daily (total daily dosage: 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) (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) Current retreatment regimens are: (iii) a fixed dose double combination of sofosbuvir/ledipasvir (400mg/90mg) once a day plus ribavirin twice a day
Study Hypotheses	**(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 (ii) Adjunctive ribavirin improves cure rates with biomarker-stratified short-course and fixed duration DAA first-line regimens (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.
Primary Outcome Measure	For the varying duration comparison the primary outcome will be: ▪ Sustained Virological Response (SVR, plasma HCV RNA persistently <LLOQ (lower limit of quantification)) measured 12 weeks after the end of the combined first and any re-treatment phases (SVR12) For the ribavirin comparison the primary outcome will be: ▪ SVR12 after first-line treatment only
Secondary Outcome Measure(s)	▪ SVR12 after first-line treatment (where not the primary outcome) ▪ SVR12 after the end of the combined first and any re-treatment phases (where not the primary outcome) ▪ SVR24 after the end of the combined first and any re-treatment phases ▪ SVR24 after first-line treatment only ▪ lack of initial virological response ▪ viral load rebound after becoming undetectable ▪ serious adverse events ▪ grade 3/4 adverse events ▪ grade 3/4 adverse events judged definitely/probably related to interventions ▪ treatment-modifying adverse events (any grade) ▪ grade 3/4 anaemia ▪ emergence of resistance-associated HCV variants ▪ sensitivity/specificity of point–of-care diagnostic for IL28 ▪ costs and cost-effectiveness

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Randomisation	Patients will be allocated 1:1 using a factorial design to each of • biomarker-stratified varying vs fixed duration • adjunctive ribavirin or not Randomisation will be stratified.
Number of Patients to be Studied	408
Duration	▪ Patients are planned to be recruited over 2 years ▪ Each first-line intervention will be administered for 4-8 weeks ▪ 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 ▪ The overall trial duration is planned for 4 years (including start-up and close-out)
Sponsor	Imperial College London
Funder	Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership (14/02/17)
Trial Manager	Emily Dennis
Chief Investigator	Graham Cooke
MRC CTU at UCL Project Leader	Ann Sarah Walker

5 TRIAL SCHEMA

Figure 1:



6 TRIAL ASSESSMENT SCHEDULE

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

		DAY POST RANDOMISATION*					EOT	WEEK POST EOT			
	SCREENING†	0	3	7	14	28		4	8	12	24
Control: 8 weeks treatment [continuing]		DAA	[DAA]	[DAA]	[DAA]	DAA		*(see retreatment schedule below for any treatment after first-line EOT)*			
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[a]		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[b]		X					X			X	
EDTA blood for haematology[c,h,i] (5ml)	(X)	X			X	X	X			X	X
Clotted blood for biochemistry[d,h,i] (5ml)	(X)	X			X	X	X			X	X
Coagulation markers (2.5ml)	(X)										
Real-time HCV viral load[h,i] (10ml)	(X)	X	X	X	X	X	X	X	X	X	X
Point of care IL28 polymorphism test (Epistem)[e, g]		X									
Total blood draw in ml for real-time tests	-	20	10	10	20	22.5	22.5	10	10	22.5	22.5
If HIV-infected - HIV viral load (9ml) (additional) - CD4 cell count [f]	(X) (X)						X (X)				X (X)

() 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 ±1 day of the nominal visit day and end of treatment (EOT) visits within ±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 log10 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[1] (SF-12, version 2) and the Cognitive Function Scale[2] (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 [a,b] (2.5ml)	X									
Whole blood in PAXgene blood RNA tube (Qiagen) [a] (2.5ml)	X									
Storage: PBMC (20ml) [c,d]	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[(a)]	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[(b)]	X				X			X	
EDTA blood for haematology[(c,g,h)] (5ml)	(X)	X	X	X	X			X	X
Clotted blood for biochemistry[(d,g,h)] (5ml)	(X)	X	X	X	X			X	X
Coagulation markers[(g)] (2.5ml)	(X)								
Real-time HCV viral load[(g,h)] (10ml)	(X)	X	X	X	X	X	X	X	X
Storage: sites processing all samples locally									
EDTA plasma for storage[(g,h)] (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
If HIV-infected - HIV viral load (9ml) (additional) - CD4 cell count	X (X)				X (X)				X (X)

* 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[1] (SF-12, version 2) and the Cognitive Function Scale[2] (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>coagulation markers>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 x 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 specimens – 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

- 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 Blood collection tubes
- Pre-printed Thermal Transfer Labels: For use on 2.0mL Cryovials and Specimen Log forms
- Forms: STOP HCV-1 Lab Request forms Specimen Storage and Shipping Logs

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

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

9 SPECIMEN LABELS

9.1 LABELLING BLOOD COLLECTION TUBES

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.

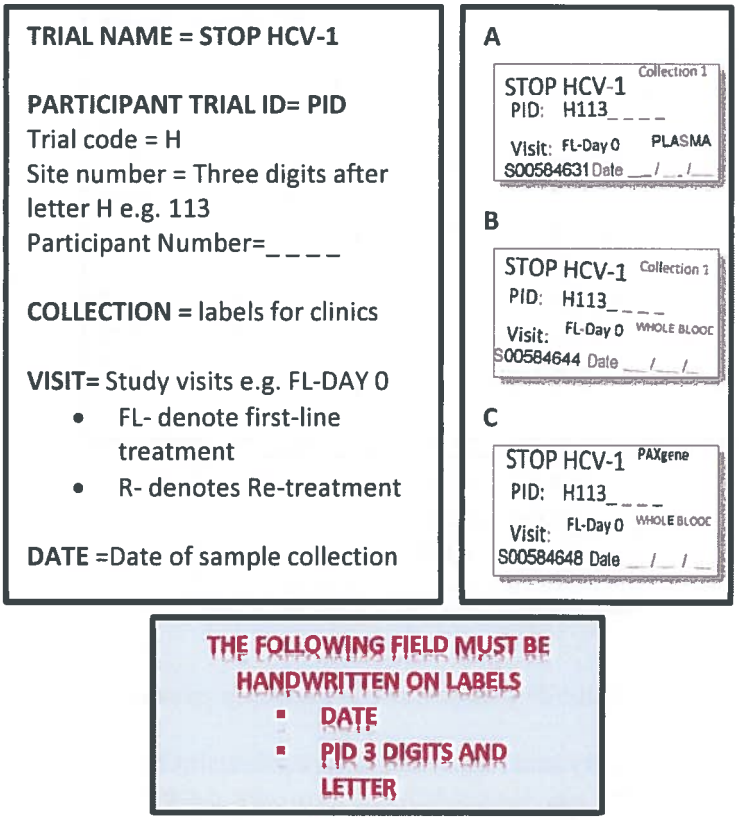


Figure 1: Understanding specimen collection labels

- 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
- The Laboratory Request Form (**Appendix II**) should accompany the specimens to the lab (duplicate this forms if necessary).

9.2 LABELLING CRYOVIALS

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.

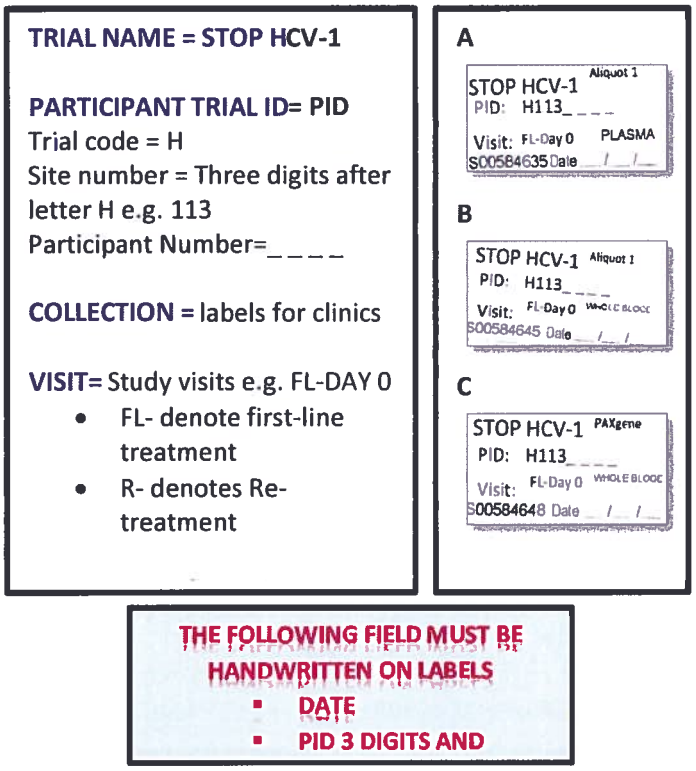
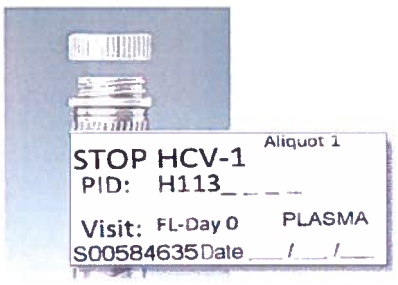


Figure 2: Understanding sample processing labels

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

- 7x7 cryobox labelled OXPAX001 = PAXgene tubes stored at -80^{0}C
- 9x9 cryobox labelled OXWBL001 = Whole Blood stored at -80^{0}C.
- 9x9 cryobox labelled OXPLA001 = Plasma all time points stored at -80^{0}C
- 9x9 cryobox labelled OXFL001 = FL-Day 0 2x Plasma aliquot stored at -80^{0}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 specimen 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 COMPLETING THE STOP-HCV-1 SPECIMEN LOGSAMPLE TRACKING

- Specimens should be logged on the STOP-HCV-1 electronic specimen log (**Appendix I**).
- Each sample 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 2nd 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: FIRST LINE (FL) TREATMENT SPECIMEN COLLECTION

Visit	Specimen	Type of Collection Container	Volume of Specimen Collected	Stored as	Type of storage container
FL-Day 0	Plasma	EDTA Blood Collection Tube	**20 ml**	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
FL-Day 3	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-Day 7	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-Day 14	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-Day 28	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-EOT	Plasma	EDTA Blood Collection Tube	**20 ml**	6 x 1 ml	Cryovial
FL-EOT+4	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-EOT+8	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
FL-EOT+12	Plasma	EDTA Blood Collection Tube	**20 ml**	6 x 1 ml	Cryovial
FL-EOT+24	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.**

- 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.
- 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)
- Participants randomised to varying durations (between 28-49 days) will have specimens collected depending on the number of days.
- If the treatment duration falls on exactly FL-Day 28 then this is defined as FL-EOT

TABLE 2: RE-TREATMENT SPECIMEN COLLECTION

Visit	Specimen	Type of Collection Container	Volume of Specimen Collected	Stored as	Type of storage container
R-Week 0*	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-Week 2	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-Week 4	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-Week 8	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-EOT	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-EOT+4	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-EOT+8	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-EOT+12	Plasma	EDTA Blood Collection Tube	10 ml	3 x 1 ml	Cryovial
R-EOT+24	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 until it is centrifuged. Within 4 hours of collection blood is centrifuged then immediately aliquoted.
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 cryovials.
7) Immediately freeze for storage in 81 grid box and document the position on the STOP-HCV-1 specimen log (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 tab of the specimen log).

12.2 COLLECTION OF BLOOD FOR WHOLE BLOOD (FOR 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 (Appendix I)

12.3 COLLECTION OF BLOOD FOR WHOLE BLOOD (FOR 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 tab on the specimen log (**Appendix I**)

13 STOP-HCV-1 SPECIMEN SHIPPING PROCEDURES

13.1 SHIPPING STOP-HCV-1 SAMPLES FROM SITES TO SPECIMEN REPOSITORY

Samples processed locally at sites remain at sites until STOP-HCV-1 team contact to centralise the samples in Oxford or Imperial College London.

Prior to shipment (locally stored samples) sites should ensure that:

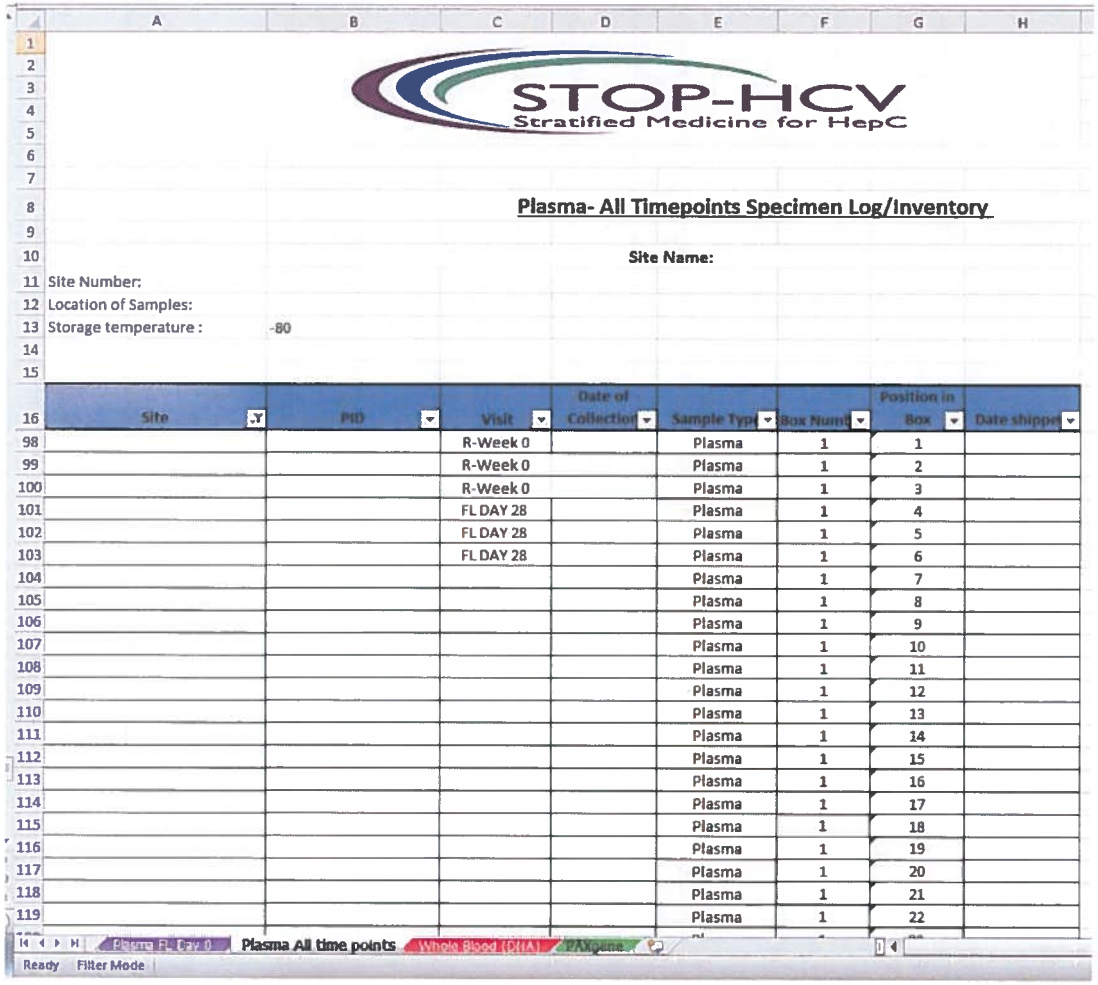
- The specimens are correctly labelled and the position of the specimen in the box matches the position indicated on the log.
- The specimen log emailed to the STOP-HCV-1 Coordinating Centre before the shipment date.

On the day of the shipment:

- Please make sure all specimens and paperwork are completed correctly
- Place a print out of the 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 LOG



STOP-HCV
Stratified Medicine for HepC

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 Num	Position in Box	Date shipped
		R-Week 0		Plasma	1	1	
		R-Week 0		Plasma	1	2	
		R-Week 0		Plasma	1	3	
		FL DAY 28		Plasma	1	4	
		FL DAY 28		Plasma	1	5	
		FL DAY 28		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	1	16	
				Plasma	1	17	
				Plasma	1	18	
				Plasma	1	19	
				Plasma	1	20	
				Plasma	1	21	
				Plasma	1	22	

Plasma FL Day 0 | Plasma All time points | Whole Blood (RNA) | PAXgene

Ready Filter Mode

APPENDIX II STOP HCV-1 LAB REQUEST FORM

MRC | UCL

STOP-HCV
Stratified Medicine for HepC

Attach Participant ID label

Laboratory Request Form
Version 1.0 16-Mar-2016

Visit Date: d d m m m y y y y

First line Day ☐☐ EOT ☐
Retreatment Week ☐☐ EOT ☐

☞ 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

A. PLASMA

Date Specimen obtained: d d m m m y y y 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	Number of aliquots stored
☐☐ • ☐ ml	☐☐	☐☐

☞ Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels

B. WHOLE BLOOD (DNA ISOLATION)

Date Specimen obtained: d d m m m y y y y

2.5 ml Required at First Line Day 0

Collected in EDTA tubes — **Stored**

Size of collection tubes	Number of tubes collected	Number of aliquots stored
☐☐ • ☐ ml	☐☐	☐☐

☞ Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels

B. WHOLE BLOOD (RNA ISOLATION)

Date Specimen obtained: d d m m m y y y y

2.5 ml Required at First Line Day 0

Collected in PAXgene tubes — **Stored**

Size of collection tubes	Number of tubes collected	Number of PAXgene tubes stored
☐☐ • ☐ ml	☐☐	☐☐

☞ Ensure all tubes/cryovials are labelled with STOP HCV-1 study specific labels

Samples sent by:

Signature:	Printed Name:	Date Completed: d d m m m y y y y

Samples processed by:

Signature:	Printed Name:	Date Completed: d d m m m y y y y

☞ Place aliquots into corresponding grid box and document on Specimen Log with STOP HCV-1 Specimen Log Label.

STOP-HCV-1 Lab Form v1.0 16-Mar-2016 — Page 1 of 1

APPENDIX III: SITES PROCESSING SPECIMENS LOCALLY

- 019-Swansea, Singleton Hospital
- 022-Leicester, Royal Infirmary
- 057-St Mary's , Imperial
- 080-London, St. George's Hospital
- 095-Nottingham, Queens Medical Centre
- 103-Guildford, Royal Surrey County Hospital
- 113-Brighton, Royal Sussex County Hospital
- 142-Oxford, John Radcliffe Hospital
- 302-Newcastle, Freeman Hospital
- 420-London, Mortimer Market Centre