LABORATORY MANUAL

HDClarity (UCL-CHDI)
Version: 5.0
05 January 2021

Contents

1 GENERAL CONSIDERATIONS

! Robust local procedures must be in place to ensure a plentiful supply of antiseptic applicators - BD CareFusion Chloraprep 3mL, catalogue no. 260400. Your contract includes the cost for a case of 100 of these. You must order a case with the latest expiration date available.

1.1 STORAGE

! Ensure that there is a robust institutional policy on freezer failure that includes checks, alarms, emergency contact details, backup power supplies, CO2 cylinders and an infrastructure to transfer samples to an off-site facility if necessary.

- Place all samples per participant (e.g. each individual sample set) in the Biohazard bag during storage. If the biohazard bag precludes efficient storage, samples may be stored in racks and/or other bags as long as they are stored together and clearly identifiable. The bags must be kept for later shipping.

- Samples will be stored at site for a minimum of 3 months and until you have at least 5 participant samples and will then be shipped in bulk to BioRep. Samples to be shipped include CSF, Cells from CSF, Serum, and Plasma.

2 BIOKITS

2.1 INFORMATION

! NOTE To obtain the highest quality specimens possible, all collection supplies should be stored at room temperature before use.

Each BioKit label is pre-printed with an internal BioRep code, the **Kit ID**, which is to be its primary source of identification. **Please do not swap labelled supplies between kits** as they are all linked together using this code and swapping them around could cause subject samples to be mixed up. If for any reason any Kit-ID-labelled supplies are moved between kits, then you must inform HDClarity Central Coordination ('HDClarity CC', HDClarity-CC@enroll-hd.org) of the details and clearly document this on the Electronic Data Capture system (EDC).

Please check expiration dates on the individual kit items before using the kit and use older kits first to prevent expiry. The following items have expiration dates:

- Vacutainer blood collection tubes
  - Lithium heparin tubes
  - Serum tube
- Sterile wound pack
- Sterile gauze and swabs
- Syringes
- Needles
- Sterile 1ml polypropylene pipette

If any item has expired, please dispose only of the expired items and mark the kit as incomplete. HDClarity CC will provide replacement items or further instructions. Please do not dispose of the whole kit when an item expires.

Please carefully manage your biokit ordering to synchronize with your estimated recruitment needs, and request further kits on the EDC in advance of running out. Also be aware of expiration dates in relation to the recruitment schedule at your site, if you have kits that will expire prior to your next sampling visit then please contact HDClarity CC with the Kit IDs.

WarmMark Temperature Tags:

Before use, the WarmMark temperature tags should be stored at room temperature. On the day that the shipment is scheduled (by emailing BioRep), two inactivated temperature tags per shipment box should be placed in the -80C freezer. On the day of shipment (about a week later), be sure to activate the temperature tags and when the samples are packed into the dry ice shipping box, place one in the biohazard document pouch of a kit at the bottom of the shipping box and one at the top of the shipping box. More details on this process can be found in section 7.1 and 7.3.

2.2 CONTENTS

Each Biokit contains the following four sub-kits: (Plus a Biohazard bag for all samples to be placed in for shipping)

Lumbar Puncture sub-kit	1) 1 x Sterile dressing pack 2) 5 x Sterile gauze swabs (10x10cm) 3) 2 x 10ml syringe 4) 2 x 25g hypodermic needle, (1 inch) 5) 2 x 18g blunt drawing up needle (1.5 inches) 6) 2 x 21g hypodermic needle 7) 2 x Spinal needle: 24g x 3.5 (purple) 8) 2 x Spinal introducer needle: 20g x 1.25 (yellow) 9) 3 x 20ml aspiration syringe for CSF collection 10) 1 x Styrofoam cup 11) 3 x 50ml polypropylene collection tube. Labelled 'CSF Collection Tube'. 12) 1 x Clear skin dressing with non-adherent pad 13) 1 x Big plastic bag (30 x 40cm approx) or equivalent 14) 1 x Note for site ("study site to supply 2 chloraprep swabs")	**Blood Collection sub-kit**	1) 1 x Disposable tourniquet 2) 3 x Alcohol wipes 3) 2 x 21g butterfly needle 4) 1 x Vacutainer barrel 5) 1 x Vacutainer adapter 6) 1 x Cottonball 7) 2 x Small round plaster 8) 4 x Lithium heparin tube (10ml, including labels) 9) 1 x Serum tube (8.5ml, including label) 10) 1 x Small plastic bag (10x15cm approx) or equivalent 11) 1 x Medium plastic bag (15x25cm approx) or equivalent
CSF Processing sub-kit	1) 2 x Sterile polypropylene Pasteur pipettes (3ml) 2) 1 x Cardboard tube for PP pipettes 3) 1 x 30ml polypropylene tube labelled 'CSF Supernatant' 4) 5 x individually wrapped sterile polypropylene pipette tips (1ml) 5) 75 x 0.5ml polypropylene cryovial, labelled 'CSF', sterile w/blue tops 6) 1 x 0.5ml polypropylene cryovial, labelled 'Cells from CSF', sterile w/yellow tops 7) 1 x RNA*later* stabilization solution (1.5ml aliquot) 8) 1 x 96-well rack for cryovials. Labelled 'CSF'. 9) 1 X Bulb 10) 1 x Medium plastic bag (15 x 25cm approx) or equivalent	**Blood Processing sub-kit**	1) 2 x Sterile polypropylene Pasteur pipettes (3ml) 2) 1 x 50ml polypropylene tube. Labelled 'Plasma'. 3) 5 x Individually wrapped sterile polypropylene pipette tips (1ml) 4) 3 x 2ml polypropylene cryovial, labelled 'Serum', sterile w/clear tops 5) 75 x 0.5ml polypropylene cryovial, labelled 'Plasma', sterile w/red tops 6) 1 x 96-well rack for cryovials. Labelled 'Plasma'. 7) 1 x Bulb 8) 1 x Medium plastic bag (15x25cm approx) or equivalent 9) 1 x Small plastic bag (10x15cm approx) or equivalent
Storage Materials	1) 1 x Absorbent material 2) 1 x Biohazard bag (33x46cm approx) or equivalent (including label) 3) 1 x Big plastic bag (30x40cm approx) or equivalent 4) 1 x WarmMark temperature tag (tag should be stored at room temperature until use)		

3 SAMPLE COLLECTION

3.1 LABORATORY SCHEDULE FOR HDCLARITY

VISIT	SCREENING	SAMPLING	OPTIONAL REPEAT SAMPLING	RESCREENING[1]
DAY	-30 to -1	0	28 to 56	
FULL BLOOD COUNT (FBC)	X			X
CLOTTING PROFILES: Prothrombin Time (PT) AND Activated Partial Thromboplastin Time (APTT)	X			X
C-Reactive Protein (CRP)	X			X
SERUM SAMPLE		X	X	
PLASMA SAMPLES		X	X	
CSF/Cells from CSF		X	X	

[1] as required

3.2 SCREENING VISIT

3.2.1 SAFETY LABORATORY TESTS

There is no remote lab testing for HDClarity safety samples. Your Local accredited clinical laboratory (i.e. ISO 15189 compliant or equivalent) will carry out routine tests on the safety blood samples taken during the Screening Visit: Full Blood Count, Clotting Profiles (PT and APTT) and CRP. The upper and lower limits for the test results are defined by your local laboratory. These limits and the actual value of the test result should be entered in the EDC (Screen shot 1).

If a participant's blood count or clotting profiles are outside the ranges below, then they are excluded, unless the result is deemed not clinically significant and a waiver is granted by the Chief Investigator. In the event of an abnormal laboratory result that the site PI has reason to believe will improve within the screening window, participants may be rescheduled for a repeat screening visit to have these safety laboratory tests repeated, if the Chief Investigator gives approval.

3.2.2 ANALYTES AND ACCEPTABLE RANGES

Full Blood Count

White Cell Count	(within normal range)
Neutrophil Count	(within normal range)
Lymphocyte Count	(within normal range)
Hemoglobin	(within normal range)
Platelets	(within normal range)

Clotting Profiles

Prothrombin Time	(within normal range)
Activated Partial Thromboplastin time	(within normal range)

CRP < 2 × upper limit of normal

The local lab must receive the samples within 6 hours in order to test the specimens

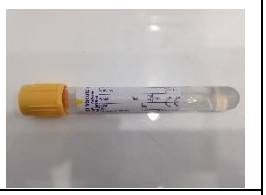
3.2.3 SAFETY LABORATORY SAMPLE COLLECTION

For the Safety laboratory assessments (Full Blood Count, Clotting and CRP) use local tubes and venipuncture equipment. Samples will be processed locally.

! Please make sure that all caps are tightly secured.

! Check the expiration date on the tube – do not use expired tubes!

! Your local tubes may vary from those shown – check with your local clinical lab and HDClarity CC if there is any doubt

Specimens are best collected through venipuncture using a butterfly needle vacuumed directly into the required tube.	
CRP Gold top 5ml tube This sample should be drawn first	
Full Blood Count Lavender top 4ml tube This sample should be drawn second	
Clotting profiles: PT and APTT Blue top 4.5ml tube Special care is needed for collecting and handling blood samples intended for coagulation testing. If sample clotting or hemolysis occurs during collection the test cannot be performed. This sample should be drawn last and then mixed by gentle inversion 8-10 times.	

3.2.4 INSTRUCTIONS FOR COMPLETING THE REQUISITION FORMS

Blood samples for safety lab testing will be sent to your local laboratory and a local requisition form should be completed and sent along with the samples to the lab.

Full blood count, Clotting: PT and APTT and CRP are essential blood tests at screening and must always be requested along with any other tests that the PI or delegate thinks are necessary for checking the safety of the participant to join the study.

3.3 SAMPLING VISIT

3.3.1 CSF COLLECTION

! Robust local procedures must be in place to ensure a plentiful supply of antiseptic applicators (BD CareFusion Chloraprep 3mL, catalogue no. 260400), Lidocaine, wet ice and dry ice.

CSF SAMPLE COLLECTION PREPARATION – BEFORE PARTICIPANT ARRIVAL

1. Check kit contents and availability as specified in section 2.2
2. Check equipment which you will provide yourselves:
   Lidocaine
   Dry Ice
   Wet Ice
   Antiseptic applicators
3. Check expiration dates on all equipment
4. Pre-cool one centrifuge to 4°C ready for CSF and plasma
5. Ensure availability of second centrifuge ready for serum processing at room temperature
6. The gold standard is for parallel processing of samples using 2 centrifuges as described in points 4 and 5. If this is not possible then you can process the samples in series instead, prioritising the CSF, then plasma and then serum.
7. Pre-cool 50 ml CSF collection tubes x3 on wet ice
8. Stock transfer container with wet ice for transferring sample to the lab
9. Stock dry ice container
10. Pre-cool the cryovials and processing tubes
11. Prepare a sterile field containing all equipment needed
12. Ensure all tubes are correctly labelled

PRE-LUMBAR PUNCTURE ASSESSMENT

Log in to the HDClarity Sampling or Optional Repeat Sampling electronic case report form (eCRF), complete the pre-procedure checks; Eligibility Check and Checklist SMP, and enter the source data online, to ensure safety and eligibility, before proceeding to lumbar puncture.

LUMBAR PUNCTURE

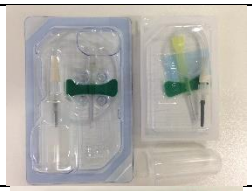
1. Identify L4/5 or L3/4 space using surface markings (i.e. the intercristal line)
2. The LP and CSF collection can be performed with the participant in either the lateral decubitus or sitting position, according to your local preferred clinical practice. This should be discussed with the participant and they should be allowed to choose the alternative position if they prefer. The participant may also be transferred to the alternative position during the procedure to aid CSF collection.
3. Disinfect skin using antiseptic applicator.
4. It is highly recommended to use adequate lidocaine to reduce the discomfort of this LP procedure. If, after noting allergies or sensitivities to lidocaine and discussing the risks and benefits of local anaesthesia, it is decided to forgo this step, it should be noted in the case report form. Inject up to 5ml of 2% lidocaine for local anaesthesia. Use the 25G 1" needle and inject lidocaine to raise a skin wheal. Then inject lidocaine more deeply using the 21G needle. If the participant is thin, do not insert the deep infiltration needle all the way. Use only about 2/3 of its length to prevent entering the subarachnoid space with anything other than the pencil-point spinal needle.
5. The spinal introducer needle should be placed along the intended angle of injection and advanced until in position.
6. Introduce 24G atraumatic spinal needle with opening facing rostrally.
7. If CSF cannot be obtained, up to three attempts are allowed. An alternative design of spinal needle supplied by the site may be used if, after at least one attempt with the supplied needle, it is felt this will increase the chance of success.
8. If the CSF collection fails, then there is no need to collect blood samples from the participant at this visit.
9. An adjacent space may be used (with further lidocaine, max. total 10 ml, if needed).

10. Once CSF is seen, attach a 20ml collection syringe and with gentle negative pressure, collect the first 1ml of CSF into the syringe and check the first 1ml for blood staining. If the first 1 ml is not macroscopically bloody, continue sampling CSF in the same syringe.
11. Document the space and position used for lumbar puncture, the number of needle passes (i.e. the number of times a needle is inserted and removed from the skin), the number of attempts (i.e. the number of times the lumbar space, the participant position, or the investigator conducting the LP change), the technique used (i.e. negative pressure or drip), the volume of lidocaine used, and the time CSF collection started and ended in the eCRF.
12. Omit pressure measurement for all subjects (this is because polypropylene manometers are not available).
13. CSF can be collected with or without suction. If collected with suction, the 20ml collection syringes provided in the kit should be used. Syringes should be connected to the needle. If collected without suction, use the 50ml tubes placed on wet ice in the Styrofoam cup. If the first 1 ml is macroscopically bloody,
    - Stop collecting CSF by reinserting the stylet partially.
    - Discard the syringe or tube, and collect a second 1 ml in a new syringe or pre-cooled 'CSF' tube, and examine it visually for blood contamination.
    - If it is free of blood, continue collecting CSF up to 14-19 ml (1ml less than the locally permitted maximum).
    - If the second separately collected ml of CSF is also macroscopically bloody, discard the syringe or tube, and continue to collect 13-18 ml of CSF in a third new syringe or pre-cooled 'CSF' tube.
    - If the third tube is macroscopically bloody, stop collecting and abandon the procedure or attempt the LP in a different space, if there is reason to believe blood-free CSF can be obtained. You may need to open a new collection kit to provide sufficient syringes or tubes; if this creates any discrepancies in the kit ID numbers, it must be noted carefully and explained in the eCRF.
    - Stop collecting CSF when sampling time exceeds 20 minutes. Document these details in the eCRF.
14. Place cap on tube and leave on wet ice until further processing.
15. Reinsert the stylet before withdrawing the needle.
16. Cover the puncture site with sterile dressing.
17. Record time of CSF collection (time when CSF was first seen).

18. At the discretion of the Site Principal Investigator, participants may be instructed to lie flat for 1 hour.

Transport CSF immediately to laboratory for processing, do not wait for the blood samples to be ready as this can cause delays.

3.3.2 PLASMA AND SERUM COLLECTION

! Please make sure that all caps are tightly secured. ! Check the expiration date on the tube – do not use expired tubes! ! Do not collect blood samples if CSF collection was not successful!	
Specimens are best collected through venipuncture using a butterfly needle vacuumed directly into the required tube.	
1. Fill 4 x 10 ml blood in lithium heparin tubes	
2. Gently invert each lithium heparin tube 10 times immediately after collection, and place on wet ice	
3. Fill 1 x 8.5ml serum tube	
4. Immediately after collection transfer all blood samples to the lab for processing	

4 SAMPLE PROCESSING

4.1 CENTRIFUGE INSTRUCTIONS

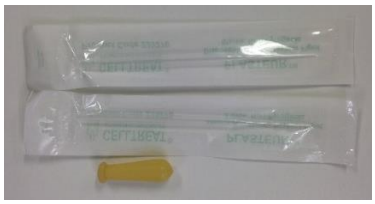
- Evidence of annual centrifuge calibration must be provided at the site initiation visit
- Your centrifuge must have accurate temperature control and clear instructions for pre-chilling
- If the number of suitable centrifuges you have available at your site limits parallel processing of CSF, plasma and serum, then samples must be processed in the following order of priority: CSF first, then plasma, then serum
- For all centrifugations, do not use the brake function but allow slow deceleration

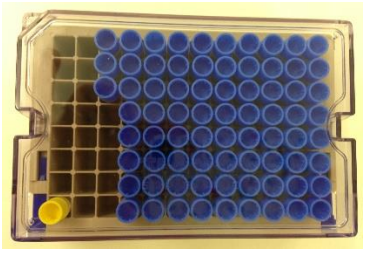
Calculate the speed setting (rpm) that corresponds to a centrifugation force of 400g, 1300g, and 2000g for the corresponding centrifuge models as required. The centrifugation force depends on rotor radius and centrifugation speed (rpm) and therefore changes if the rotor is changed. For more information and help for calculation, please visit these webpages:
https://www.sciencegateway.org/tools/rotor.htm and/or
http://www.endmemo.com/bio/grpm.php

4.2 SPECIMEN PROCESSING PROCEDURES FOR HDClarity

4.2.1 CSF PROCESSING

Sample Collection	1. Lab to receive one 50ml CSF collection tube filled up to 20mls with CSF (collected from participant between 08:00 - 10:30 local time) 3 tubes are provided in case of blood contamination. All clean CSF sent to the lab should be in a single tube.	
	2. CSF sample is collected while the collection tube is in the styrofoam cup filled with wet ice. Sample is transported to the lab in wet ice (container to be supplied by site).	
	3. Samples transported immediately to laboratory for processing.	 **Processing must start within 15 minutes of sample collection**
	4. After CSF collection, details including the Kit ID are recorded in the **CSF** eCRF, 'CSF collection' box (Screen shot 2).	
Sample Processing	5. Note the CSF processing start time.	
	6. Agitate the entire CSF sample for 10 seconds using a vortex mixer to homogenise CSF.	
	7. Using a sterile individually wrapped polypropylene 1ml pipette tip, extract 200 µl of the CSF and use it to determine white blood cell count and	

	erythrocyte count per µl in triplicate according to local GLP-approved laboratory practice as instructed at the site initiation visit and in the Manual CSF Cell Count SOP. Cell counts should be recorded on the 'CSF Quality' eCRF in the 'Onsite CSF Sample Quality Control' box (Screen shot 3).	  **Triplicate cell count should be done within 60 minutes of sample collection.**
	8. Balance the centrifuge and before filling the balance tube with water please clearly mark the tube so that it can easily be identified as water (not CSF). 9. Centrifuge the 50ml tube containing residual CSF at **400 × g for 10 min at 4°C** to remove cells while preserving cell integrity for potential future use. Cell integrity is needed so that intracellular substances do not contaminate the non-cellular phase of the CSF.	**Label your balance tube!**
	10. Using the polypropylene Pasteur pipette, transfer the supernatant into a single 30ml polypropylene tube labelled 'CSF supernatant' and agitate for 10 seconds to homogenise CSF. If the polypropylene Pasteur pipettes are damaged it is acceptable to decant the supernatant into the tube. No pipettes should be used other than those supplied.	 

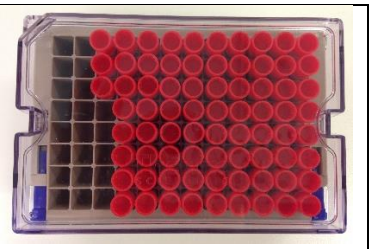
	Aliquot the CSF in 300 µl aliquots into the pre-cooled cryovials labelled 'CSF' on wet ice, using a sterile individually wrapped polypropylene 1ml pipette tip. Note the tube rack ID, tube ID (this must be the same for all aliquots) and the number of aliquots for later recording on the eCRF. Please dispose of any unused cryovials. CSF aliquots must have blue lids. Any samples that do not have the expected lid colour will be discarded by BioRep.	
	11. Re-suspend the CSF cell pellet in 300 µl of supplied RNA*later* solution, using gentle vortex agitation, and use another sterile pipette tip to transfer to a cryovial with yellow lid labelled 'Cells from CSF'. **Dispose of empty vials – Do not ship or re-use them.**	 
Sample Storage and Shipment	12. Immediately after processing freeze CSF aliquots and the resuspended cells in your -80°C freezer. Ensure samples are stored upright and all lids are secure. Plasma, Serum and CSF do not need to be stored in the freezer at the same time – if waiting for the blood to be ready will cause a delay, then store the CSF in the freezer first, rather than waiting. If there will be any delay in getting the samples into the freezer then they can be kept in dry ice for a short period of up to 5 minutes. Please document this	FREEZE AT -80°C AND SHIP AFTER A MINIMUM OF 3 MONTHS, AND WHEN YOU HAVE AT LEAST 5 SAMPLES

	on the worksheet or source notes to explain how the samples were stored if not transferred immediately to the freezer.	
	Details of CSF processing are recorded on the **CSF** eCRF, 'CSF processing' box (Screen shot 2). Record the following parameters: Start time of CSF processing End time of CSF processing CSF tube rack ID CSF aliquot tube ID and number of cryovials Cells from CSF tube ID Date and time the samples are stored Any discrepancies in ID must be explained bearing in mind the ID is the only way to reconcile samples with participants.	  

4.2.2 PLASMA SAMPLE PROCESSING

Sample Collection	1. Gently invert each tube 10 times immediately after collection, and place on wet ice.	
	2. Samples transported immediately to laboratory for processing.	 Processing must start within 15 minutes of sample collection
	3. Lab to receive 4 x 10 ml blood in lithium heparin tubes.	
Sample Processing	4. Note the following for later entry into the eCRF, or enter directly: Lithium heparin tube IDs Plasma aliquot tube ID Start time of plasma processing	 
	5. Spin lithium heparin tubes at **1300×g for 10 min at 4°C** immediately on arrival.	
	6. Discard any tubes whose plasma is pink due to haemolysis. In the unlikely event that they are all pink then use all of the tubes but clearly label the sample as contaminated.	
	7. Combine the supernatant in one tube labelled 'Plasma' and mix by inverting 10 times. Place on wet ice.	
	8. Aliquot the plasma in 300 µl aliquots into the pre-cooled cryovials labelled 'Plasma' on wet ice, using a sterile individually wrapped polypropylene 1ml pipette tip.	Dispose of empty vials – do not ship!

<table>
<tr><td></td><td>Plasma aliquots must have red lids. Any samples that do not have the expected colour lid will be discarded by BioRep.

Dispose of empty vials – Do not ship or re-use them</td><td>  </td></tr>
<tr><td rowspan="2">Sample Storage and Shipment</td><td>9. Freeze samples on dry ice and store at -80°C

Ensure samples are stored upright and all lids are secure</td><td rowspan="2">FREEZE AT -80°C AND SHIP AFTER A MINIMUM OF 3 MONTHS, AND WHEN YOU HAVE AT LEAST 5 SAMPLES</td></tr>
<tr><td>10. Record the following on the ‘Blood Processing’ tab in the eCRF (Screen shot 4):

LiHep tube ID
Processing start time
Plasma aliquot tubes ID
Plasma aliquot tube count
Time plasma processing is completed
Time of frozen storage (if serum and plasma times of freezing are different then it is the time of freezing the plasma which is most important to record in the EDC)</td></tr>
</table>

4.2.3 SERUM SAMPLE PROCESSING

Sample Collection	1. Lab to receive 1 x 8.5ml filled serum tube	
	2. Samples transported immediately to laboratory for processing.	 Processing must start within 15 minutes of sample collection
Serum Sample Processing	3. Note the following for later entry into the eCRF, or enter directly: Serum aliquot tube ID Start time of serum processing	
	4. Spin serum tube at **2000×g at room temperature for 10 minutes** immediately on arrival	
	5. Using a sterile individually wrapped polypropylene 1ml pipette tip, transfer 1500 µl of the supernatant into each of 2 of the cryovials with clear lids labelled 'Serum'. Only 2 serum cryovials are required. **Dispose of empty vials – Do not ship or re-use them**	
Sample Storage and Shipment	6. Freeze cryovials on dry ice and then store in -80°C. Inside small plastic bag alongside the CSF cells. Ensure samples are stored upright and all lids are secure.	FREEZE AT -80°C AND SHIP AFTER A MINIMUM OF 3 MONTHS, AND WHEN YOU HAVE AT LEAST 5 SAMPLES
	7. Record the following on the 'Blood processing' eCRF (Screen shot 4): Serum aliquot tube ID Start time of serum processing Serum aliquot tube count Time processing was completed Time of frozen storage (if serum and plasma times of freezing are different	

	then it is the time of freezing the **plasma** which is most important to record in the EDC)	

5 SAMPLE QUALITY CONTROL MEASURES

	Measured	Cut-off for flagging
Microscopic erythrocyte count in CSF	In triplicate - locally	> 1000 erys/µl
Microscopic leukocyte count in CSF	In triplicate - locally	≥ 5 cells/µl
Hb, albumin and quantitative immunoglobulins levels in acellular CSF	Central lab	

! Please note!

'erys' (erythrocytes) is synonymous with 'cells' or 'RBCs'

AND

1 µl = 1 cubic millimetre = 1 mm^3 = 1 cumm

5.1 Manual CSF cell count

200 µl of the CSF (or amount agreed by HDClarity CC) should be used to determine white cell count and erythrocyte cell count per µl according to local GLP-approved laboratory practice. This should be done in triplicate within 60 minutes of collection and all values recorded in the CRF. We strongly encourage sites to use a manual cell count procedure as described below, as automatic techniques have been proven to lead to inaccuracies (Kleine et al., Clin Chem Lab Med. 2010 Jun;48(6):839-48.)

5.1.1 MATERIALS

- Fuchs-Rosenthal chamber and coverslips
- Pipette

5.1.2 METHOD

- Pipette 200 µl CSF and allow to warm to room temperature
- Slightly pre-wet the cover slip mounting support of the Fuchs-Rosenthal chamber using a small amount of distilled water or just by breathing against it

- Attach the cover slip by firmly pushing it over the cover slip mounting support (the cover slip should firmly adhere to the counting chamber; Newton's rings should appear between both mounting supports and the cover slip)
- Gently mix CSF using a pipette to ensure even distribution of the cells
- Fill the pipette with 10 µl cell suspension
- Place the pipette tip close to the glass cover edge, right at the centre of the chamber
- Release the plunger slowly, checking that the liquid enters the upper part of the chamber uniformly, being absorbed by capillarity
- If bubbles appear, or the glass cover has moved, repeat the operation after cleaning the chamber
- Let the cells in the cell counting chamber sediment for 2-3 minutes
- Count cells under the microscope using 200x magnification and using a cell counter
- Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits, which should not be taken into account
- Identify erythrocytes as disc-shaped cells without nucleus, whereas leukocytes have inner structures identifiable as nucleus (see Figure 1).
- Count erythrocytes and leukocytes separately and count all erythrocytes and leukocytes in the whole field of 256 squares
- Fill the lower section of the chamber with newly suspended CSF as above and repeat counting after sedimentation
- Clean cell counting chamber and repeat counting as above a third time

Calculation: The conversion factor depends on the technique (i.e. the counting chamber used). It is recommended that you follow the manufacturers guidelines (Fuchs-Rosenthal chamber - Number of cells counted/3.2 = cells/µl).

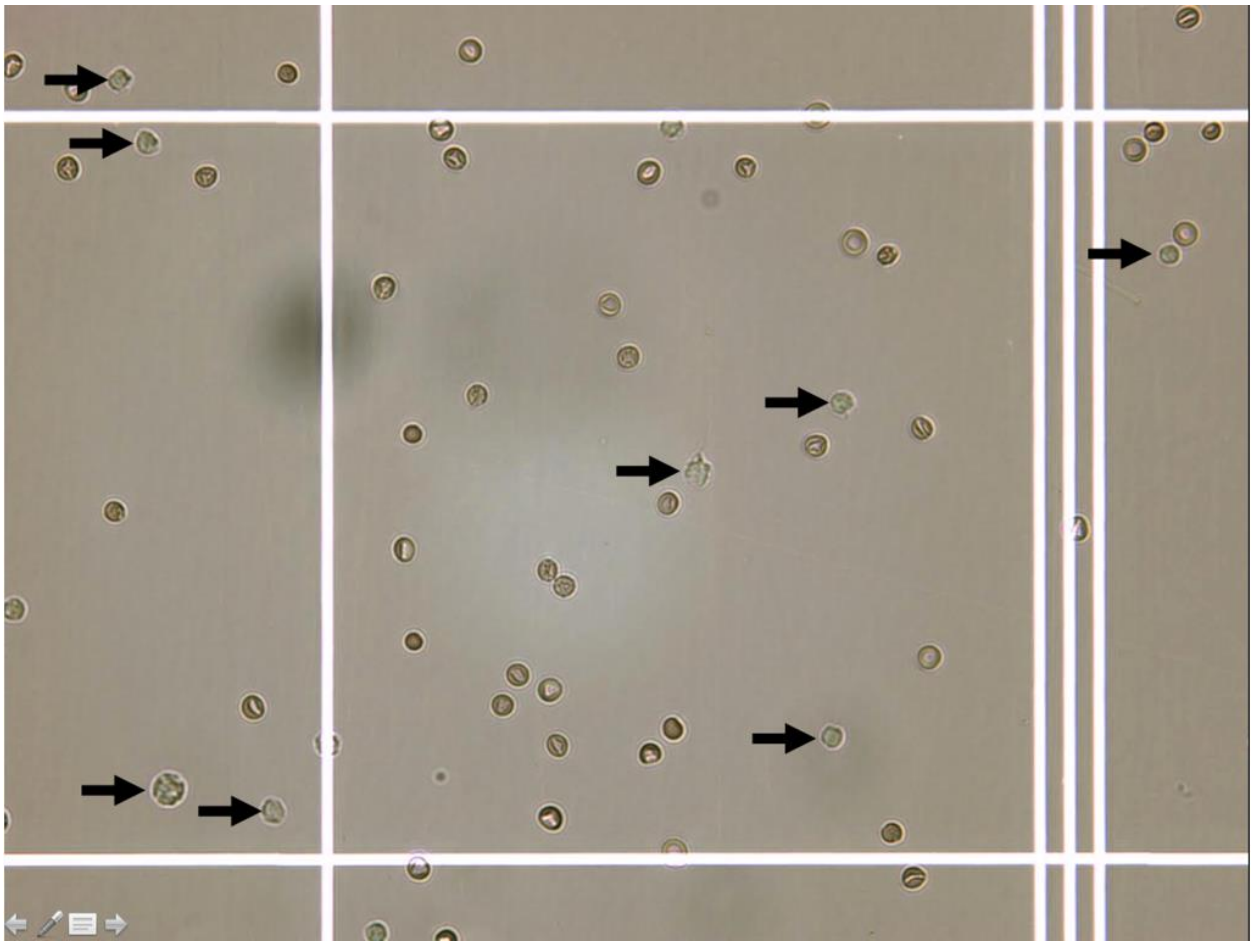


Figure 1: Micrograph of CSF with blood contamination observed with phase contrast microscopy. Leukocytes are marked with arrows.

6 ELECTRONIC DATA CAPTURE

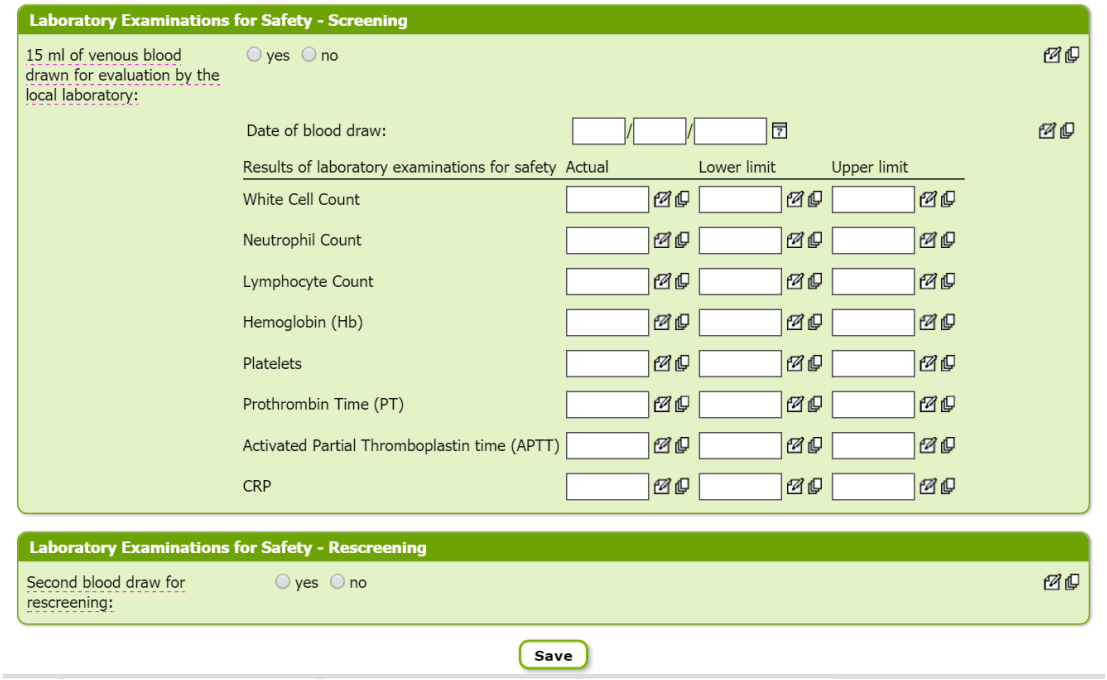
There are two options for ensuring that your data is captured accurately on the EDC: 1) direct data entry or 2) your site can create source worksheets to record the data and then enter on to the EDC later (data must be entered on to the EDC to trigger site payments).

Screening Visit

Following the screening visit or repeat screening visit one CRF relating to sample collection will become available on the EDC; 'Safety Lab Exam'.

You must enter the results of the laboratory examinations as well as your local lab ranges on the EDC.

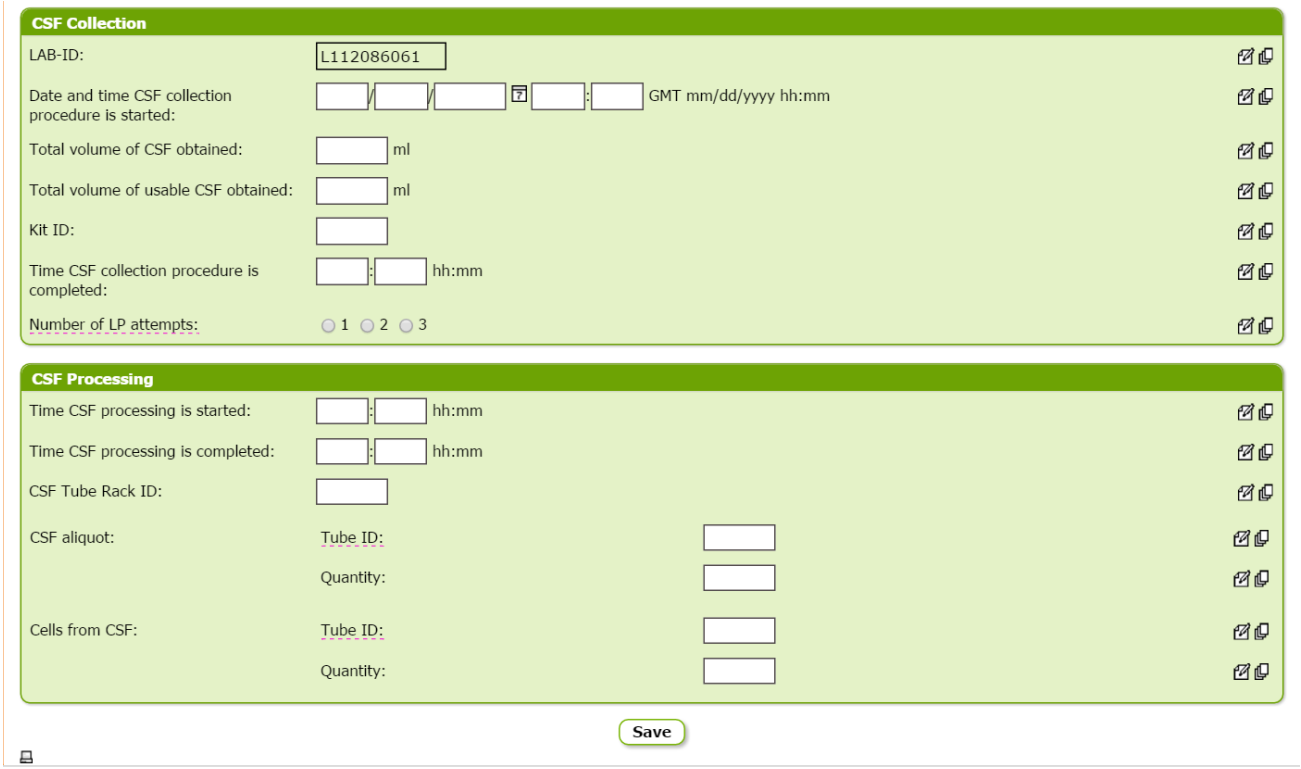
Screen shot 1:



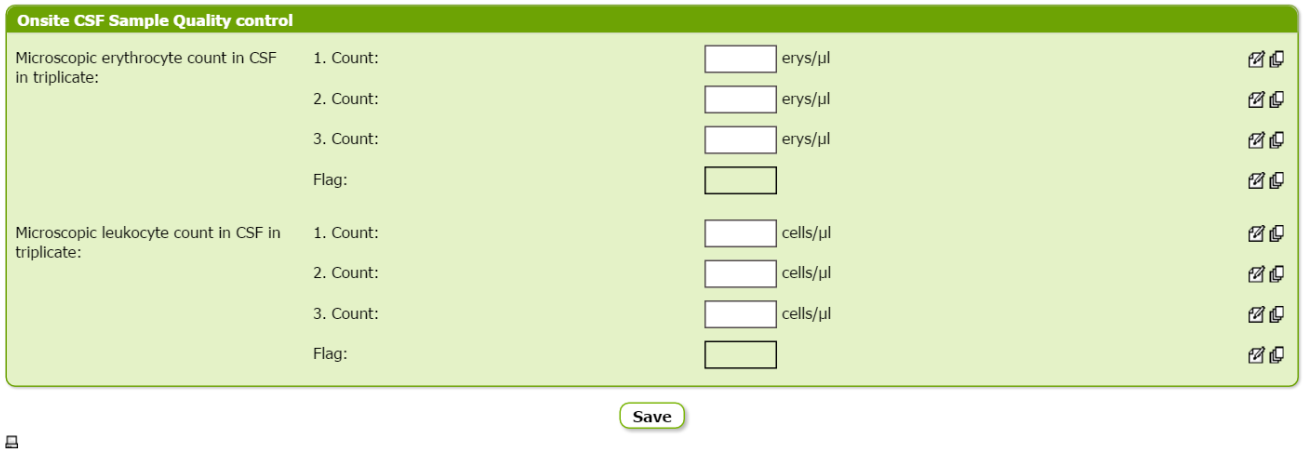
Sampling Visit

During the Sampling or Repeat Sampling Visit three tabs relating to biosamples will become available: CSF (collection and processing), CSF Quality and Blood Processing.

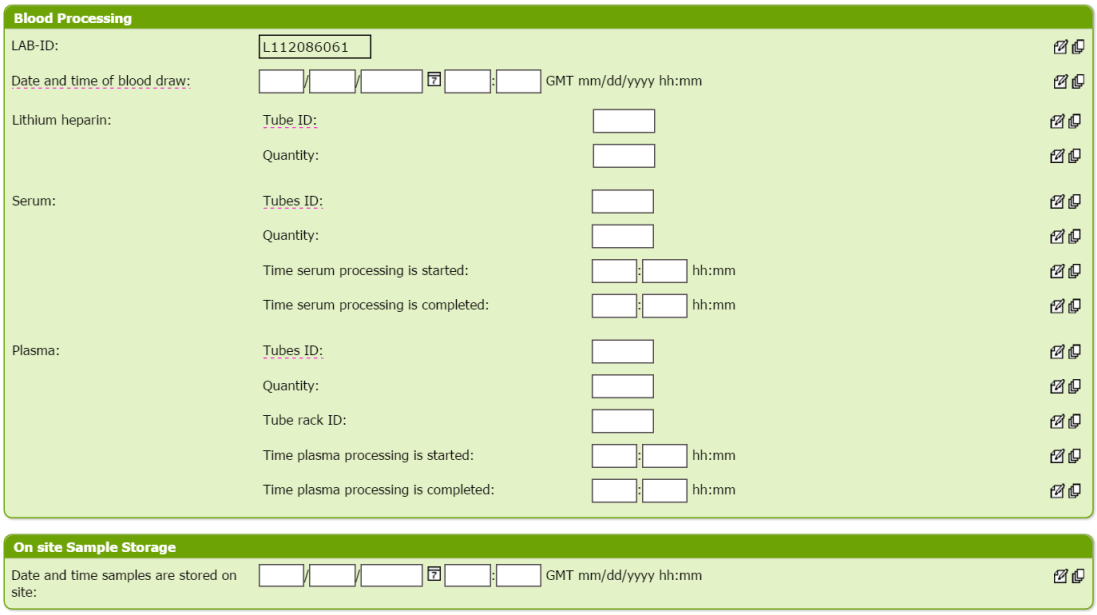
Screen shot 2: CSF



Screen shot 3: CSF Quality



Screen shot 4: Blood Processing


Blood Processing
LAB-ID:
L112086061
Date and time of blood draw:
GMT mm/dd/yyyy hh:mm
Lithium heparin:
Tube ID:
Quantity:
Serum:
Tubes ID:
Quantity:
Time serum processing is started:
hh:mm
Time serum processing is completed:
hh:mm
Plasma:
Tubes ID:
Quantity:
Tube rack ID:
Time plasma processing is started:
hh:mm
Time plasma processing is completed:
hh:mm
On site Sample Storage
Date and time samples are stored on site:
GMT mm/dd/yyyy hh:mm
Save

_

7 SHIPMENTS AND KIT ORDERING

7.1 SHIPMENTS REQUIREMENTS

World Courier or Marken will be used for this study and will provide packaging to transport samples to BioRep (including dry ice).

1. Contact BioRep hdclarity@biorep.it to schedule a collection. **You must give BioRep at least 3-5 working days-notice for scheduling pick-up** Prepare the samples the same day BioRep is contacted to schedule sample pick-up: • Ensure each sample set is in one biohazard bag • Place two ***inactivated*** WarmMark temperature tags per shipment in the -80ºC freezer to await the scheduled shipment date • Ensure that there are 2 temperature tags for every 5 samples (the goal is to have a shipment of 5 samples, with 2 temperature tags per shipment)
2. Samples should be shipped to BioRep after a minimum of 3 months and when you have at least 5 participant samples. The only exception to this is if you have reached the capacity of your freezer or if directed to ship samples by HDClarity CC, when a smaller box can be provided. Please aim to fill each shipment box. If you are unsure of which shipment boxes to use, please ask BioRep or HDClarity CC to help you order the correct size of box.
3. Plan to ship on Mondays, to avoid problems with weekend delays and to avoid transit and hold in airport during the weekend (but note the 'public holidays' section)
4. The courier will provide a polyfoam box/s, dry ice (enough for 3 days), and a temperature monitoring device (only if WarmMark temperature tag is unavailable).
5. All sites must complete the 'No X-ray' form and sites outside of Europe must also complete the 'proforma invoice' form provided to you by HDClarity CC (see the 'Shipping Documents' section).

6. Once you have contacted BioRep to schedule the collection the courier will call you on the contact details you have provided. During this call please confirm the following:
    a. Quantity of sample sets you are shipping.
    b. Type and quantity of boxes you will need (a box size GDI 30 will accommodate 5 participant samples). [1]
    c. Dry ice is required.
    d. Time and date of collection.
    e. World Courier or Marken AWB number (sometimes referred to as the HAWB).
    f. Who the contact person is at your site – make sure they have a contact phone number).
    g. Whether the courier can collect direct from the freezer.

If WarmMark temperature tags are unavailable for use, you should also request one temperature monitoring device per shipping box.

7. The courier may request a draft copy of the completed and signed 'No X-ray' form and/or the 'proforma invoice' before the collection so that they can ensure that the format and contents of the document will facilitate a smooth customs clearance.

8. **If the courier has not contacted you at least 24 hours in advance of the shipment date then please inform HDClarity CC immediately.**

9. If the courier cannot collect the samples directly from the freezer, the site must prepare a polystyrene container and dry ice to transfer samples from the freezer to the collection point.

10 When the courier arrives to collect the shipment:

    a. **Take out the pre-cooled WarmMark temperature tags from the -80°C freezer, activate them and place them in the document pouch of a biohazard bag at the bottom of the shipping box and one at the top of the shipping box.**
    b. Check that the World Courier or Marken AWB number you received over the phone matches the AWB number they have.
    c. Check the quantity of dry ice covers the samples.
    d. **If the WarmMark temperature tags are unavailable, ensure the courier switches on and places a temperature monitoring device in each box** – you will be expected to sign to say you have witnessed this.

12. Complete the shipment notification in the EDC, including the tracking number (World Courier or Marken AWB number) supplied by the courier to ensure that BioRep is aware that the shipment will be in transit. Each notification will include the following information: quantity and type of samples, the study name, the site name (and Site ID), the researcher who initiates the shipment, and the LabID.
13. You can use the EDC to track which shipments are at the site, shipped or arrived and if there are any damaged or missing.
14. When samples are received at BioRep, BioRep uploads the samples and the Bulk Shipment form is updated. BioRep will enter the quantity, arrival status and the arrival date at BioRep to the Bulk Shipment form.
15. BioRep will provide a summary report to the CHDI Biorepository Manager and study team indicating if the sample went below 0°C at any point during the shipment, as well as pictures of each WarmMark temperature tag.

[1] Smaller boxes are available, but should only be used in exceptional circumstances, e.g. if directed by HDClarity CC or if your freezer is full and you don't have more participants scheduled to use a 5-participant box.

7.2 PUBLIC HOLIDAY CONSIDERATIONS

Local courier service (pick-up and delivery) may be limited prior to, during and following observed holidays in your country and in the country to which you are shipping specimens. It is imperative that you check local service schedules in advance of the holidays.

Ship samples to BioRep after a minimum of 3 months and when you have at least 5 participant samples, or as instructed by HDClarity CC. Shipping must only be done on Mondays, to avoid weekend delays. The package should contain sufficient dry ice for three days.

Listed below are important considerations when planning your participant visits which fall in or near to holidays.

- Your courier service reserves the right to observe earlier than usual pick-up times during the holidays. Please check with your courier service for local pick-up schedules.
- Please schedule your pick-ups in advance of the holiday where possible.
- Contact BioRep as early in the day as possible to schedule your pick-up.

- Samples should NOT be shipped on the day before an observed holiday, please wait to ship on the next available business day.

- For sites with 24 hours delivery time to BioRep, do not schedule any shipment 24 hours before a holiday.

- For sites with 48 hours delivery time to BioRep, do not schedule any shipment 48 hours

7.3 PACKAGING PROCEDURES

<table>
<tr>
<td><u>To Be Completed When Shipping Date is Scheduled:</u>

Place the following into the Biohazard bag provided:

Rack containing all cryovials labeled ‘CSF’

Rack containing all cryovials labeled ‘Plasma’

Small bag containing all cryovials labeled ‘Serum’ and single cryovial labeled ‘Cells CSF’

Remove the white plastic from the top of the bag and then fold the bag over so that the black square is placed over the star, and then seal the bag.

Place two inactivated WarmMark temperature tags in the -80ºC freezer until shipment.

Place sealed biohazard bags back in the -80C freezer.</td>
<td>Biohazard Bag containing samples and WarmMark temperature tag ready for shipment.

  </td>
</tr>
<tr>
<td colspan="2"><u>To Be Completed on Day of Shipment:</u></td>
</tr>
</table>

When the courier arrives, they will place the biohazrad bag/s onto dry ice and then place in a polyfoam box. Up to 5 bags may be shipped in any single shipment box (boxes and dry ice provided by World Courier or Marken).

If using the WarmMark temperature tag, make sure each temperature tag is activated before the shipping box is sealed. Temperature tag activation requires the top edge of the tag to be folded and pulled.

Make sure there are two WarmMark temperature tags is in each shipment box – one in the document pouch of a bag at the bottom of the shipment box and one at the top of the shipment box.

If WarmMark temperature tags are not available and you are using a temperature monitoring device supplied by the courier, ensure that the courier activates the device and places it in the box with the samples.

7.4 SHIPPING DOCUMENTS

The courier will provide packaging and the prefilled AWB.

All sites must complete the 'No-X-ray' form (Appendix 1) before the courier arrives to collect the samples. You must provide the courier with three copies of the form – all signed by the person at the site who has arranged the shipment. HDClarity CC will provide you with an electronic copy of this form and you must insert your site letter head to it. All sections highlighted must be completed.

Sites outside of Europe must also complete the 'Proforma Invoice' form (Appendix 2) before the courier arrives to collect the samples. You must provide the courier with nine copies of the form and three of these must be signed by the person at the site who has arranged the shipment. HDClarity CC will provide you with an electronic copy of this form and you must insert your site letter head to it. All sections highlighted must be completed.

! Please note that where it states PatID on the Proforma Invoice this refers to the KitID which is on all of the bags within the kit. No participant IDs should be on/with the shipped samples.

! Please note that sites within the United Kingdom will now need to complete a 'Proforma Invoice' form for shipments after January 1, 2021.

After the courier has collected the samples, complete the shipment notification in the EDC to ensure that the BioRep is aware that the shipment is now in transit. Each notification will include the following information:

- Quantity and type of samples
- Study name
- Site name (and Site ID)
- Name of researcher who initiates the shipment
- LabID

Once you have it then enter the World Courier or Marken AWB number of the bulk shipment container into the EDC.

7.5 ORDERING BIOKITS

1. Additional HDClarity sample kits can be requested at any time via the biokit ordering form on the Enroll-HD website.

2. The order form must be completed with the following details, which should appear automatically based on log-in credentials:

   Name of site

   First and last name of requester

   Requester's email address

   Requester's phone number

   Postal address where biokits should be delivered to

   BioRep will automatically receive the Site ID on the notification

3. If the transmission is successful, the request order number will appear on the form with a message reading 'Your order has been successfully sent to the biorepository at BioRep and was registered under order number XXX'.

4. You cannot order a subcomponent of the kit.

5. Your local supply should not drop below 5 kits unless recruitment has been paused; if you have fewer than 5 participant screening visits planned, you need sufficient kits for at least the number of planned screenings.

6. Older kits should be used first to prevent expiry.

7. Any unused tubes should be discarded by your site.

8. Robust local procedures must be in place to ensure a plentiful supply of lidocaine 2% and antiseptic applicators (BD CareFusion Chloraprep 3mL, catalogue no. 260400).

8 DANGEROUS GOODS TRAINING REQUIREMENTS

All site staff involved in HDClarity will be trained on the protocol with special attention given to training in the collection, handling, processing, QC and shipping of the CSF and blood samples.

HDClarity CC will train sites using the training materials and in-person training if deemed necessary. Your site will be certified following satisfactory completion of training and certification requirements.

There is a document repository within the EDC which hosts HDClarity training videos and training manuals. The repository location will appear in the EDC: https://studies.enroll-hd.org/training

The site Principal Investigator is responsible for ensuring their staff have completed the required training and meet their local regulations.

Sites must establish and maintain a record of your staff's training and date of the instruction.

It is necessary for all individuals involved in the preparation or transport of dangerous goods to be properly trained and tested initially, with follow-up training as mandated by local standards and regulations. Additional training updates are required any time the applicable regulations change.

9 SAMPLE DESTRUCTION REQUEST

In the event a participant or legally authorized representative of a participant withdraws consent to retain samples, the Biosample Destruction Request Form (Appendix 4) will need to be completed for HDClarity samples tested or stored at BioRep. An electronic version of this form will be supplied to you by HDClarity CC. Please email this form to HDClarity CC who will then get authorization from CHDI and submit a withdrawal notice to BioRep.

10 CONTACT DETAILS

For enquiries then please use the following contacts:

hdclarity-cc@enroll-hd.org	Gail Owen Seema Maru Kat Schubert Filipe Rodrigues Alex Lowe
ITSupport@Enroll-HD.org	IT issues
hdclarity@biorep.it	To Schedule sample shipments
hdclarity-mm@enroll-hd.org	Medical Monitor
hdclarity-kitorders@enroll-hd.org	Kit Order Requests or queries

In all urgent email correspondence relating to samples please include 'HDClarity Biosamples Urgent' in the title and include your direct contact details including a contact telephone number in the email so we can respond to you as quickly as possible.

11 TRAINING DOCUMENTS

There are two training videos on the EDC listed under a specific HDClarity Study Materials and Training section. The first demonstrates CSF sample collection at UCL, a second demonstrates CSF processing.

12 APPENDICES

Appendix 1 - No-Xray Form

>>Letterhead of your site<<

Date **>>Date of Shipment <<**

Senders: **>>Name of person and Name of site<<**

Consignee **:** **BioRep srl**
c/o DIBIT2 Palazzina San Michele
Via Olgettina 60
20132 Milano Italy
Ref: Stefania Michelini
Phone: +300258014369

Object: Security Control Exemption

World Courier or Marken AWB Number: >> World Courier/MarkenAWB Number<<

Master AWB Number: >>Master AWB Number<<

I underlined >>Name<< **as** >>Designation<< **certify that the shipment Master AWB** >>MAWB Number<< **contains Blood and CSF (Biological samples) for use in the research study HDClarity (UCL-CHDI).**

We declare that shipment MAWB >>Master AWB number<< is not subjected to security control. Please do not x-ray, exposure to x-ray radiation would damage the samples.

SIGNATURE >> <<

Appendix 2 - Proforma Invoice (non-EU sites, including UK as of January 1, 2021)

>>Letterhead of your site<<

PROFORMA INVOICE

SENT BY *(inviato da)* Address: >> << *(Indirizzo)* Post Code: >> << *(CAP)* Tel N°: >> <<	**AIRBILL N°** *(Lettera di vetture N°)* >>Provided by BioRep<< **CARRIER** *(vettore):* **World Courier/Marken**
SENT TO *(inviato a)* Company *(Azienda)*: BioRep S.r.l c/o DIBIT 2 – Palazzina San Michele Address: Via Olgettina 60 *(Indirizzo)* Milano Post Code: 20132 *(CAP)* Country: Italy *(Italia)* *(Paese)* Tel / Telex N°: +39 02 58 01 43 69 VAT Registration N°: 03891970968 (*N° partita IVA*)	Number of Pieces: 1 *(N° pezzi)* Total Gross Weight (kg): >>>< kg *(Totale peso lordo)* Total Net Weight (kg): >>>< kg *(Totale peso netto)*

Full Desc. of Goods *(descrizione completa delle merci)*	**Country of Origin**	**Qty** (quantità)	**Unit value & currency**	**Sub Total Value and currency**

Please refer to the Kit ID on all of the bags and sample tubes within each kit

	(Paese d'origine)		(valore per unità e valuta)	(totale e valuta)
UN 3373 – Biological Substance Category B List of PatID: >>You must list the PatIDs of all participants whose samples are within this shipment<< Samples of: • serum (total amount of vials: ___) • plasma (total amount of vials: ___) • CSF (total amount of vials: ___) (*Campioni di siero, plasma e CSF)*	>> <<	>> How many patients<< patient sample set *(set di campioni da singolo paziente)*	>> << 1.50 € or $ / patient sample set	>> << 1.50 €/$
TOTAL VALUE & CURRENCY (valore totale e valuta)				>> << 8.50 €/$

REASONS FOR EXPORT: Research Use Only *(solo scopo di ricerca)*

(motivazioni dell'esportazione)

I declare that the above information is true and correct to the best of my knowledge *(dichiaro che l'informazione di cui sopra è vera e corretta, per quanto in mia conoscenza)*

Signature:..... Name: …… Date:……

(firma) *(nome)* *(data)*

Appendix 3 - Biosample Destruction Request Form

Please complete and send this form by email to UCL Central Coordination biosamples@hdclarity.net

Stored Specimen(s) requested to be destroyed as a Stored Specimen at BioRep under the Services Agreement

Project's name: HD Clarity	**Date of Request:** DD-MMM-YY
Reason for Destruction: >> <<	
Site ID: A-XXXX	**Site Name:** >> <<
City >> <<	**Country** >> <<
Person Submitting this Request (Name, Role) >> <<	

Lab ID#	**Kit ID**	**Date Sample Collected**	**Select the visit**
LXXXXXXXXX	XXXX	DD-MMM-YY	☐ Sampling ☐ Repeat Sampling

Approved by CHDI: Name , Title	**Date:**