LABORATORY MANUAL

HDClarity (UCL-CHDI)
Version: 6.8
26 Jan 2026

Contents

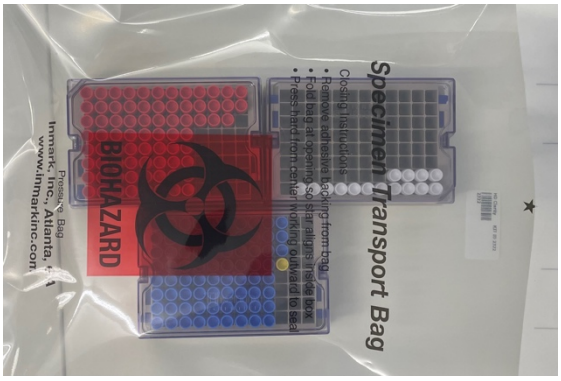
1 GENERAL CONSIDERATIONS

! Robust local procedures must be in place to ensure a plentiful supply of antiseptic applicators - BD CareFusion Chloraprep 3mL, catalogue no. 260400. 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 supplied 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 biohazard bags must be kept for later shipping.



- Samples will be stored at site until you have 4 participant sample sets or unless otherwise directed by HDClarity CC. They 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. See 2.2 Biokit Contents table for further details on kit components. Please note that the highlighted items **only** correspond to kit components that cannot be replaced locally and must be replaced by BioRep. The majority of other kit components also have expiration dates which also need to be checked before use.

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. You can also request fewer than 5 kits at a time if this more closely aligns with your site's recruitment estimates. To make this request, please reach out directly to HDClarity CC, do not place a kit order on the EDC. 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 requested (by emailing BioRep), one inactivated temperature tag per participant visit should be placed in the -80C freezer. On the day of shipment (about a week later), be sure to activate the temperature tags (by folding over and pulling out the top tab completely) and place one tag in each of the participant visit biohazard bags when the samples are packed into the shipping box with dry ice. More details of this process can be found in section 8.1 and 8.3.

2.2 BIOKIT 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) 5 x Paper cup 11) 4 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	**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 Cotton ball 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 'Cell 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) 1 x 15ml polypropylene tube. Labelled 'Serum'. 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) 2 x Medium plastic bag (15x25cm approx) or equivalent 9) 15 x 0.5ml polypropylene cryovial, labelled 'Serum', sterile w/white tops 10) 1 x 96-well rack for cryovials. Labelled 'Serum'.
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)		***Please note:** All highlighted items have expiration dates **and** must be replaced by Biorep. Replacements may be arranged through HDClarity CC.

3 SAMPLE COLLECTION

3.1 LABORATORY SCHEDULE FOR HDCLARITY

VISIT	ANNUAL SCREENING	ANNUAL SAMPLING	OPTIONAL REPEAT SAMPLING	RESCREENING[1]
DAY	-30 to -1	0	28 to 56	
URINE PREGNANCY TEST [2]	X	X	X	X
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

[2] urine pregnancy test to be performed for females of childbearing potential unless the participant is post-menopausal or not sexually active. Sites will be required to supply their own pregnancy tests.

3.2 ANNUAL 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 Annual 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 the participant will be 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 **prior** approval.

3.2.2 ANALYTES AND ACCEPTABLE RANGES

Full Blood Count

White Cell Count	(within ± 10% normal range)
Neutrophil Count	(within ± 10% normal range)
Lymphocyte Count	(within ± 10% normal range)
Hemoglobin	(within ± 10% normal range)
Platelets	(within ± 10% normal range)

Clotting Profiles

Prothrombin Time	(within ± 10% normal range)
Activated Partial Thromboplastin time	(within ± 10% normal range)

CRP	< 2 × upper limit of normal

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

3.2.3 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.

3.2.4 SAFETY LABORATORY SAMPLE COLLECTION

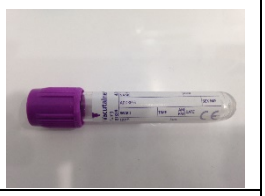
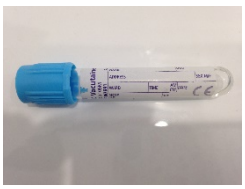
Before blood collection, ensure a negative urine pregnancy test is available for female participants (excluding post-menopausal women and those who are not sexually active) and a full neurological examination has been performed (see Appendix 1).

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.	

3.3. ANNUAL 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 ID, 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. ***Note: The serum must sit un-spun for 30 minutes to allow for clotting.** 7. Pre-cool 50 ml CSF collection tubes x4 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 on wet ice, **except yellow blood tube (serum)** 11. Prepare a sterile field containing all equipment needed 12. Ensure all tubes are correctly labelled

PRE-LUMBAR PUNCTURE ASSESSMENT
Before sample collection, ensure a negative urine pregnancy test is available for female participants (excluding post-menopausal women and those who are not sexually active) and a focused neurological exam has been performed (see Appendix 1). Log into the HDClarity Sampling (Y0, Y1, Y2 or Y3) 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. Oral sedation may be given to adolescents at the discretion of the Site Principal Investigator.
2. Identify L4/5 or L3/4 space using surface markings (i.e. the intercristal line)
3. The LP and CSF collection can be performed with the participant in either the lateral decubitus or sitting upright 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.
4. Disinfect skin using antiseptic applicator.
5. 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. Local practices should be followed for adolescents 11-18 years and 0.5% and 1% preparations are advisable to minimise toxic reactions. The maximum dose should not exceed 4.5 mg/kg (or 200mg) for participants aged 12-18 years and 3 mg/kg for those aged 11 years. 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.
6. The spinal introducer needle should be placed along the intended angle of collection and advanced until in position.
7. Introduce 24G atraumatic spinal needle with opening facing rostrally.
8. If CSF cannot be obtained, up to three attempts are allowed. An alternative design of spinal needle supplied by HDClarity CC, or one at site pre-approved by HDClarity CC, may be used if, after at least one attempt with the supplied needle, it is felt this will increase the chance of success. If there are concerns before the LP about using the supplied needle, please discuss the use an alternative needle with the HDClarity study physician.
9. If the CSF collection fails, then there is no need to collect blood samples from the participant at this visit.
10. An adjacent space may be used (with further lidocaine, max. total 10 ml, if needed). Local practices should be followed for adolescents 11-18 years. The maximum dose should not exceed 4.5 mg/kg (or 200mg) for participants aged 12-18 years and 3 mg/kg for those aged 11 years.
11. 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 up to 20 ml of CSF for participants ≥18 years of age and 5-10 ml for participants <18.

12. In the eCRF, 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 volume of lidocaine used, and the time CSF collection started and ended. ***Note:** If there is a variation in the technique used (i.e. drip instead of negative pressure), a comment should be added to the eCRF to document this. Additionally please note that the drip method should only be used if the negative pressure technique is unsuccessful.
13. Omit pressure measurement for all subjects (this is because polypropylene manometers are not available).
14. When collected with suction, the 20ml collection syringes provided in the kit should be used. Syringes should be connected to the needle. After collection, transfer the CSF from the syringe to a pre-cooled 50mL CSF collection tube. If collected without suction, use the 50ml CSF collection tubes placed on wet ice in the provided paper 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 collection tube and examine it visually for blood contamination.
    - If it is free of blood, continue collecting CSF up to 19 ml (1ml less than the locally permitted maximum) for participants aged ≥18 years and 4-9 ml for participants aged <18 years.
    - If the second separately collected ml of CSF is also macroscopically bloody, discard the syringe or tube, and continue CSF collection in a third new syringe or pre-cooled CSF collection tube; up to 18 ml for participants ≥18 years and 3-8 ml for participants aged <18 years.
    - 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 CSF collection 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.
15. Place cap on the 50ml collection tube and leave on wet ice until further processing.
16. Reinsert the stylet before withdrawing the needle.
17. Cover the puncture site with sterile dressing.

18. Record time of CSF collection (time when CSF was first seen).
19. At the discretion of the Site Principal Investigator, participants may be instructed to lie flat for 1 hour.
20. Transport the CSF in the transfer container with wet ice immediately to the laboratory for processing, do not wait for the blood samples to be ready as this can cause delays.

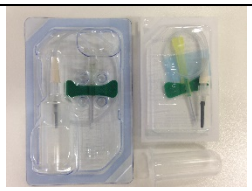
3.3.2 PLASMA AND SERUM COLLECTION

! Please make sure that all caps are tightly secured.

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

! Do not collect blood samples if CSF collection was not successful!

! There should be a maximum of 60 minutes between CSF collection ending and blood collection starting!

Specimens are best collected through venipuncture using a butterfly needle vacuumed directly into the required tube.	
1. Fill 4 x 10 ml blood in the green-topped lithium heparin tubes for participants ≥18 years and 3 x 10 ml for participants aged <18 years.	
2. Gently invert each lithium heparin tube 10 times immediately after collection, and place on wet ice.	
3. Fill 1 x 8.5ml gold-topped serum tube for all participants.	
4. Immediately after collection, transfer all blood samples to the lab for processing. The lithium heparin tubes should be transferred on wet ice and the serum tube at room temperature.	

4 SAMPLE PROCESSING

4.1 CENTRIFUGE INSTRUCTIONS

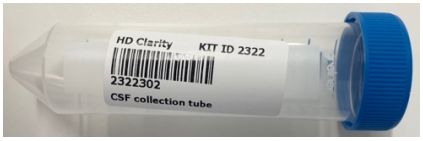
- Your centrifuge must have accurate temperature control and clear instructions for pre-chilling ***Note: CSF and plasma are processed at 4°C in a pre-chilled centrifuge. Serum is centrifuged at room temperature.**
- If the number of suitable centrifuges you have available at your site limits parallel processing of CSF, plasma, and serum, then samples must be processed in the following order of priority: CSF first, then plasma, then serum. ***Note: You must wait 30 minutes before centrifuging the serum to allow for clotting.**
- 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

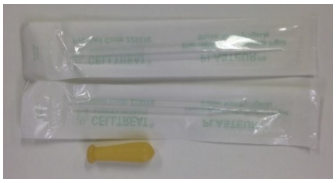
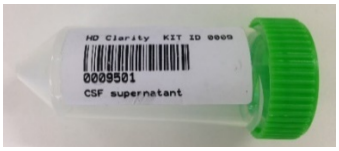
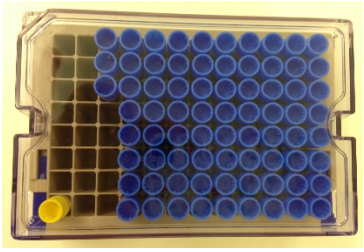
4.2 SPECIMEN PROCESSING PROCEDURES

See Appendix 5 for a summary of the key steps below for the processing of CSF and blood.

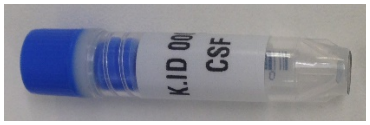
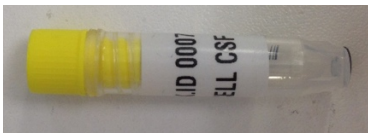
4.2.1 CSF PROCESSING

Sample Collection	1. Lab to receive **one** 50ml CSF collection tube filled up to 20mls with CSF (collected from participant after a fast of at least 6 hours) 3 extra 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 supplied paper cup filled with wet ice. Sample is transported to the lab on 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 in the 'CSF processing' box of the eCRF (Screen shot 2).	
	6. Agitate the entire CSF sample for 10 seconds using a vortex mixer to homogenise the 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).	**Label your balance tube!**

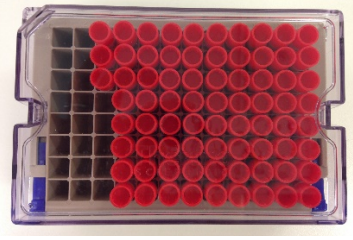
	9. Centrifuge the 50ml tube containing residual CSF at **400 × g for 10 min at 4°C, brake off** to remove cells while preserving cell integrity for potential future use. Cell integrity is needed so that intracellular substances do not contaminate the non-cellular phase of the CSF.	
	10. Using the provided polypropylene Pasteur pipette and bulb, transfer the supernatant into the single green-topped 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.	  ***Note:** Do NOT discard the blue-topped 50 ml CSF collection tube as it still contains the cell pellet.
	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. **Dispose of empty cryovials or the final cryovial if underfilled – Do not ship or re-use them** **CSF aliquots must have blue lids. Any samples that do not have the expected lid colour will be discarded by BioRep.**	 ***Tip:** Check you are aliquoting correctly by making sure the CSF is filled up to the printed black line on every cryovial. A 20ml CSF sample should produce at least 60 aliquots.

	11. Re-suspend the CSF cell pellet in 300 µl of supplied RNA*later* solution, using gentle vortex agitation or by gently pipetting the fluid repeatedly up and down. Use another sterile pipette tip to transfer the cell pellet solution to the cryovial with a yellow lid labelled 'Cell CSF' and place the cryovial in the matrix rack with the other blue lidded CSF aliquots.	  ***Note:** There should only be 1 'Cell CSF' yellow-topped cryovial. This is not the same as the 200 µl aliquot that is used for triplicate cell count (see Section 4.2.1, step 7).
	12. Note the CSF processing end time in the 'CSF processing' box of the eCRF (Screen shot 2).	CSF PROCESSING SHOULD TAKE NO MORE THAN 1 HOUR.
Sample Storage and Shipment	13. Immediately after processing, place the CSF aliquots and the resuspended cells (single yellow lid cryovial) in your -80°C freezer. Ensure samples are stored upright and all lids are secure. Plasma, Serum and CSF do not need to be transferred to the freezer at the same time –transfer the CSF to the freezer immediately, rather than waiting for the blood processing to be completed. The samples must be frozen within 5 minutes of processing ending. If there will be any delay in getting the samples into the freezer then they can be kept	FREEZE AT -80°C WITHIN 5-MINUTES OF PROCESSING ENDING AND SHIP WHEN YOU HAVE 4 PARTICIPANT SAMPLE SETS

	on dry ice for a short period of up to 5 minutes. Please document this on the worksheet or source notes to explain how the samples were stored if not transferred immediately to the freezer.	
	Make sure all the details of CSF processing are recorded on the **CSF** eCRF, 'CSF processing' box (Screen shot 2). **You should have recorded:** -Start time of CSF processing -End time of CSF processing -CSF tube rack ID -CSF aliquot tube ID and quantity (i.e., number of aliquots) -Cells from CSF tube ID -Cells from CSF quantity (should always be only 1 aliquot) -Date and time the CSF samples are stored in the -80 freezer (Note: this is recorded on the blood processing form) -Any comments regarding deviations from the processing as described above. Any discrepancies in ID must be explained bearing in mind the ID is the only way to reconcile samples with participants.	   ***Tip:** To minimize the chance of queries, record anything that deviates from the processing as described above. This could be anything from: a blood pellet noticed after centrifugation, accidentally under/overfilling cryovials, delayed start time, processing time longer than an hour etc.

4.2.2 PLASMA SAMPLE PROCESSING

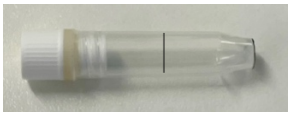
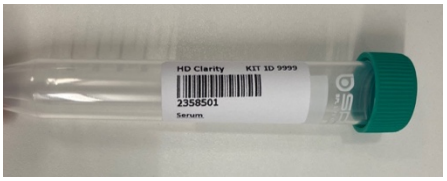
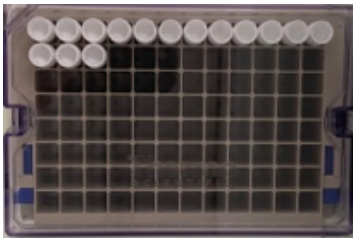
Sample Collection	1. Gently invert each tube 10 times immediately after collection, and place on wet ice.	
	2. Samples transported on wet ice immediately to laboratory for processing.	 **Processing must start within 15 minutes of sample collection**
	3. Lab to receive 4 x 10 ml blood in green-topped lithium heparin tubes (or 3 x 10 ml for participants age <18 years).	
Sample Processing	4. Note the following for later entry into the eCRF, or enter directly: -Lithium heparin tube IDs and quantity (Note: should be 4 unless participant <18 years old) -Plasma aliquot tube ID -Plasma tube rack ID	 
	5. Note the plasma processing start time in the 'Blood Processing' box of the eCRF (Screen shot 4).	
	6. Spin the lithium heparin tubes at **1300×g for 10 min at 4°C, brake off** immediately on arrival.	
	7. 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 record the sample as contaminated in the comment section of the 'Blood Processing' form on the eCRF.	
	8. Using the supplied polypropylene Pasteur pipette and bulb, combine the supernatant in one tube labelled 'Plasma', record the total volume of supernatant on the eCRF, and mix by inverting 10 times. Place on wet ice.	

	9. 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 cryovials or the final cryovial if underfilled – Do not ship or re-use them** **Plasma aliquots must have red lids. Any samples that do not have the expected colour lid will be discarded by BioRep.**	 ***Tip:** Check you are aliquoting correctly by making sure the plasma is filled up to the printed black line on every cryovial. Four lithium heparin tubes should produce at least 35 aliquots.
	10. Note the plasma processing end time in the 'Blood processing' box of the eCRF (Screen shot 4).	PLASMA PROCESSING SHOULD TAKE NO MORE THAN 1 HOUR.
Sample Storage and Shipment	11. Immediately after processing, place the plasma aliquots in your -80°C freezer. Ensure samples are stored upright and all lids are secure. The samples must be frozen within 5 minutes of processing ending. If there will be any delay in getting the samples into the freezer then they can be kept on dry ice for a short period of up to 5 minutes. Please document this on the worksheet or source notes to explain how the samples were stored if not transferred immediately to the freezer.	FREEZE AT -80°C WITHIN 5-MINUTES OF PROCESSING ENDING AND SHIP WHEN YOU HAVE 4

	Make sure all the details of plasma processing are recorded on the 'Blood Processing' tab in the eCRF (Screen shot 4): **You should have recorded:** -Lithium Hep tube ID -Lithium Hep quantity (should be 4 for adults, 3 for under 18) -Total volume of plasma transferred to 50ml tube -Plasma aliquot tube ID and quantity (i.e., number of aliquots) -Plasma aliquot rack ID -Plasma processing start time -Plasma processing end time -Any comments regarding deviations from the processing as described above. -Date and time the plasma samples are stored in the -80 freezer.	PARTICIPANT SAMPLE SETS ***Tip:** To minimize the chance of queries, record anything that deviates from the processing as described above. This could be anything from: haemolysed tubes, accidentally under/overfilling cryovials, delayed start time, processing time longer than an hour etc.

4.2.3 SERUM SAMPLE PROCESSING

Sample Collection	1. Lab to receive 1 x 8.5ml filled gold-topped serum tube	
	2. Sample transported at room temperature immediately to laboratory for processing.	
	3. Serum sample must sit un-spun (i.e., not centrifuged) at **room temperature** for **30 minutes** to allow for clotting.	 **Processing must start at least 30 minutes (and no more than 40 minutes) after sample collection** ***Tip:** Serum samples must sit at room temperature for 30 minutes while you are processing the CSF and Plasma

Serum Sample Processing	4. Note the following for later entry into the eCRF, or enter directly: -Serum collection tube ID and quantity (should be 1 because only 1x 8.5ml gold collection tube) -Serum aliquot tube ID -Serum tube rack ID	 
	5. Note the serum processing start time in the 'Blood Processing' box on the eCRF (Screen shot 4).	
	6. Spin serum tube at **2000×g at room temperature for 10 minutes, brake off.**	
	7. Using the supplied polypropylene Pasteur pipette and bulb, combine the supernatant into one green-topped 15ml polypropylene tube labelled 'Serum' and agitate for 10 seconds to homogenise the Serum.	
	8. Aliquot the Serum in 300 µl aliquots into the room temperature cryovials labelled 'Serum' using a sterile individually wrapped polypropylene 1ml pipette tip. **Dispose of empty cryovials or the final cryovial if underfilled – Do not ship or re-use them** **Serum aliquots must have white lids. Any samples that do not have the expected colour lid will be discarded by BioRep.**	 ***Tip:** Check you are aliquoting correctly by making sure the serum is filled up to the printed black line on every cryovial. One serum tube should produce at least 8 aliquots.
	9. Note the serum processing end time in the 'Blood Processing' box of the eCRF (Screen shot 4).	SERUM PROCESSING SHOULD TAKE NO MORE THAN 1 HOUR.

Sample Storage and Shipment	12. Immediately after processing, place the serum aliquots in your -80°C freezer. Ensure samples are stored upright and all lids are secure. The samples must be frozen within 5 minutes of processing ending. If there will be any delay in getting the samples into the freezer then they can be kept on dry ice for a short period of up to 5 minutes. Please document this on the worksheet or source notes to explain how the samples were stored if not transferred immediately to the freezer.	FREEZE AT -80°C WITHIN 5-MINUTES OF PROCESSING ENDING AND SHIP WHEN YOU HAVE 4 PARTICIPANT SAMPLE SETS
	Make sure all the details of serum processing are recorded on the 'Blood Processing' tab in the eCRF (Screen shot 4): **You should have recorded:** -Serum collection tube ID -Serum collection tube quantity (should be 1) -Serum aliquot tube ID and quantity (i.e., number of aliquots) -Serum aliquot rack ID -Serum processing start time -Serum processing end time -Any comments regarding deviations from the processing as described above. -Date and time the serum samples are stored in the -80 freezer (add a comment on the EDC if serum is stored at a different time than plasma)	***Tip:** To minimize the chance of queries, record anything that deviates from the processing as described above. This could be anything from: accidentally under/overfilling cryovials, delayed start time, processing time longer than an hour etc.

4.3 SAMPLE PROCESSING USING MULTIPETTE (if applicable to site)

A multipette is a manual repeating pipette (shown above on left) designed to dispense the same volume multiple times from a single aspiration and uses special tips called combitips (shown above on right).

If your site is using a HDClarity CC approved multipette and tips to process the samples, please refer to the sample processing steps in the sections above for specific volumes as well as overall required processing steps.

The general overview on using the multipette and tips though can be broken down in 10 general steps:

1. Insert the sterile combitip into the multipette. ***Note:** Push firmly so the tip is securely attached to the multipette. Do not rotate the inserted combitip to lock it in.
2. Push the filling lever down all the way.
3. Set the dispense volume to the appropriate volume (300 µl) by turning the volume selection dial until it locks into the desired position and volume amount.
4. Immerse the combitip's tip into the liquid sample.
5. Aspirate the liquid by slowly and steadily sliding the filling lever up.
6. Dispense the first one or two aliquots back into the sample collection tube by pushing the operating lever down as far as it will go. ***Note**: The first aliquot may be less than 300 µL so it should not be aliquoted into the cryovial.
7. Dispense the rest of the liquid sample into the aliquot vials by pressing the operating lever down. ***Note:** Do not dispense the liquid at an angle greater than 45° and remember to dispense the liquid slowly, as the faster the lever is pushed down, the faster the liquid will dispense.
8. Repeat step 7 until no more aliquots can be obtained. ***Note:** You may be required to repeat steps 5 and 6 if the volume is too large to be completed in one or two aspiration steps.
9. Please remove the combitip from the multipette by pushing the filling lever down and ejecting the tip over a waste container.
10. Additionally, if there is not enough sample left to use the multipette, process the final aliquots using a standard micropipette and one of the provided sterile pipette tips.

For a demonstration on how to use the multipette, please refer to the Multipette and Tips training video on the training portal. For further in-depth instructions and device specific information, please refer to your equipment's provided manual.

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

! Please note!

- 'erys' (erythrocytes) is synonymous with 'cells' or 'RBCs'
- 1 µl = 1 cubic millimetre = 1 mm^3 = 1 cumm
- No other local QC or safety tests should be performed without **prior** discussion with HDClarity CC

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 encourage sites to use a manual cell count procedure, but if your local lab routinely uses an automatic technique, this should be discussed and approved in advance with HDClarity CC prior to use. In relation, prior approval from HDClarity CC is required to perform any other variations of the above procedure, e.g. change in volume of CSF used for cell counts or fewer than three cell counts.

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).

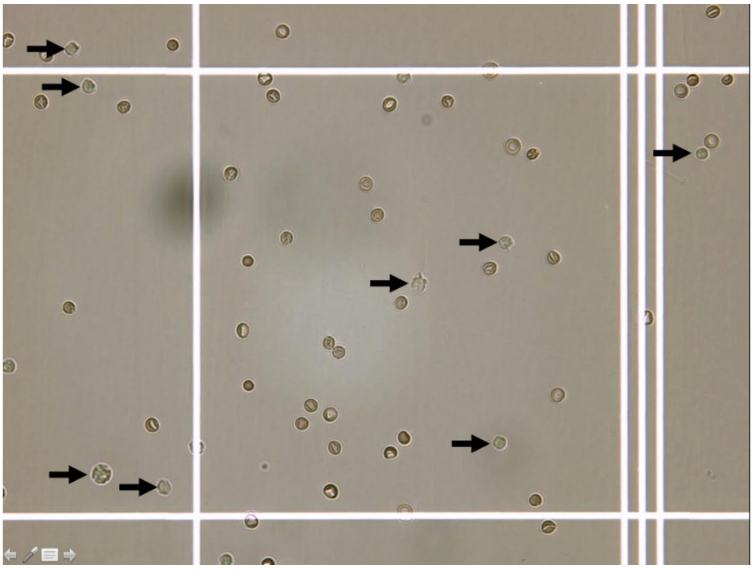


Figure 1: Micrograph of CSF with blood contamination observed with phase contrast microscopy. Leukocytes are marked with arrows.

6 EQUIPMENT

6.1 CALIBRATIONS

Evidence of regular equipment calibration must be provided for the -80°C freezer, centrifuges and pipettes that will be used for HDClarity sample processing and storage. The following must be provided at the site initiation visit and at the intervals indicated thereafter (i.e. during onsite monitoring visits):

- Pipettes are to be calibrated annually
- Centrifuges are to be calibrated annually (or as frequently as stated on the most recent calibration certificate or equipment manufacturer's manual)
- Freezers are to be calibrated every 2 years (or as frequently as stated on the most recent calibration certificate or equipment manufacturer's manual)

6.2 FREEZER TEMPERATURE LOGS

Temperature logs for the -80°C freezer where HDClarity samples are stored must be provided for review during onsite monitoring visits and the monitoring team will notify HDClarity CC of deviations >10°C for more than 60 minutes.

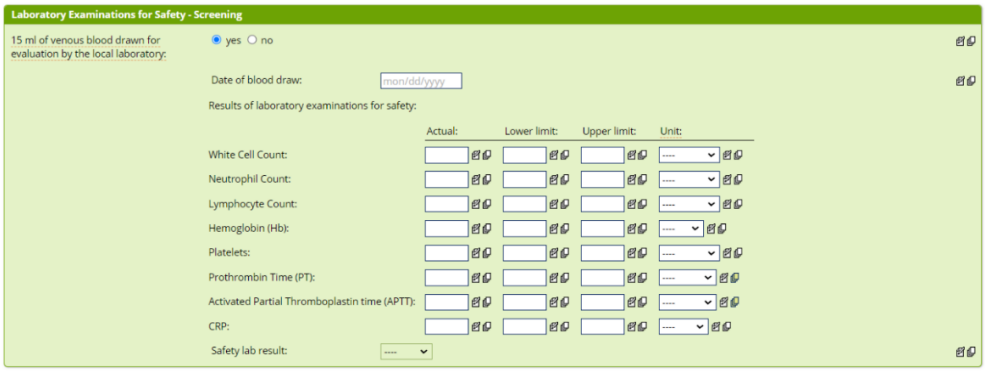
7 ELECTRONIC DATA CAPTURE

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).

Annual Screening Visit

Following the annual screening visit or a repeat screening visit, one CRF 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. The EDC will automatically allow 2 x the upper limit for CRP and ±10% variance for all other values and the result will only be classified as a "fail" if it falls outside of these ranges.

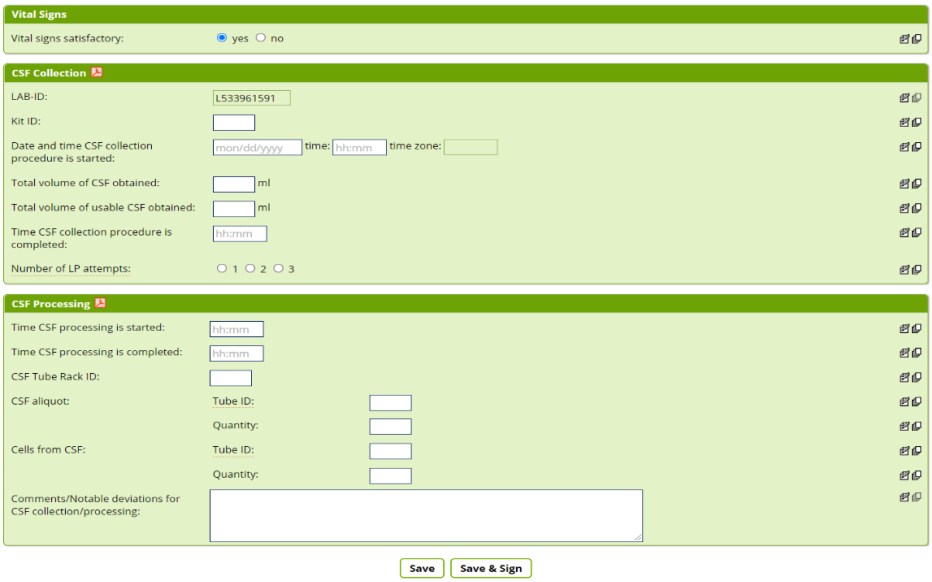
Screen shot 1: Safety Lab Exam



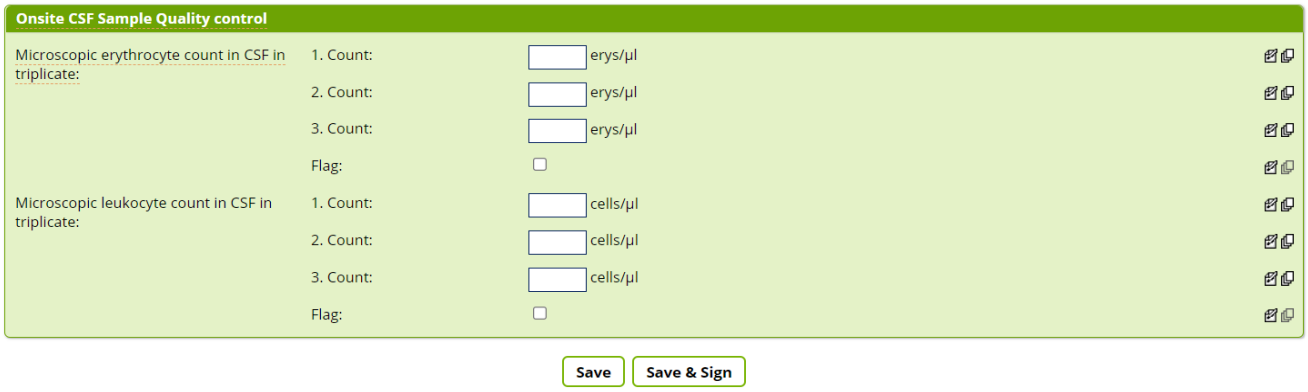
Annual Sampling Visit

During the Sampling (Y0, Y1, Y2 or Y3) or Optional Repeat Sampling Visit, three tabs relating to will become available: CSF (collection and processing), CSF Quality and Blood Processing.

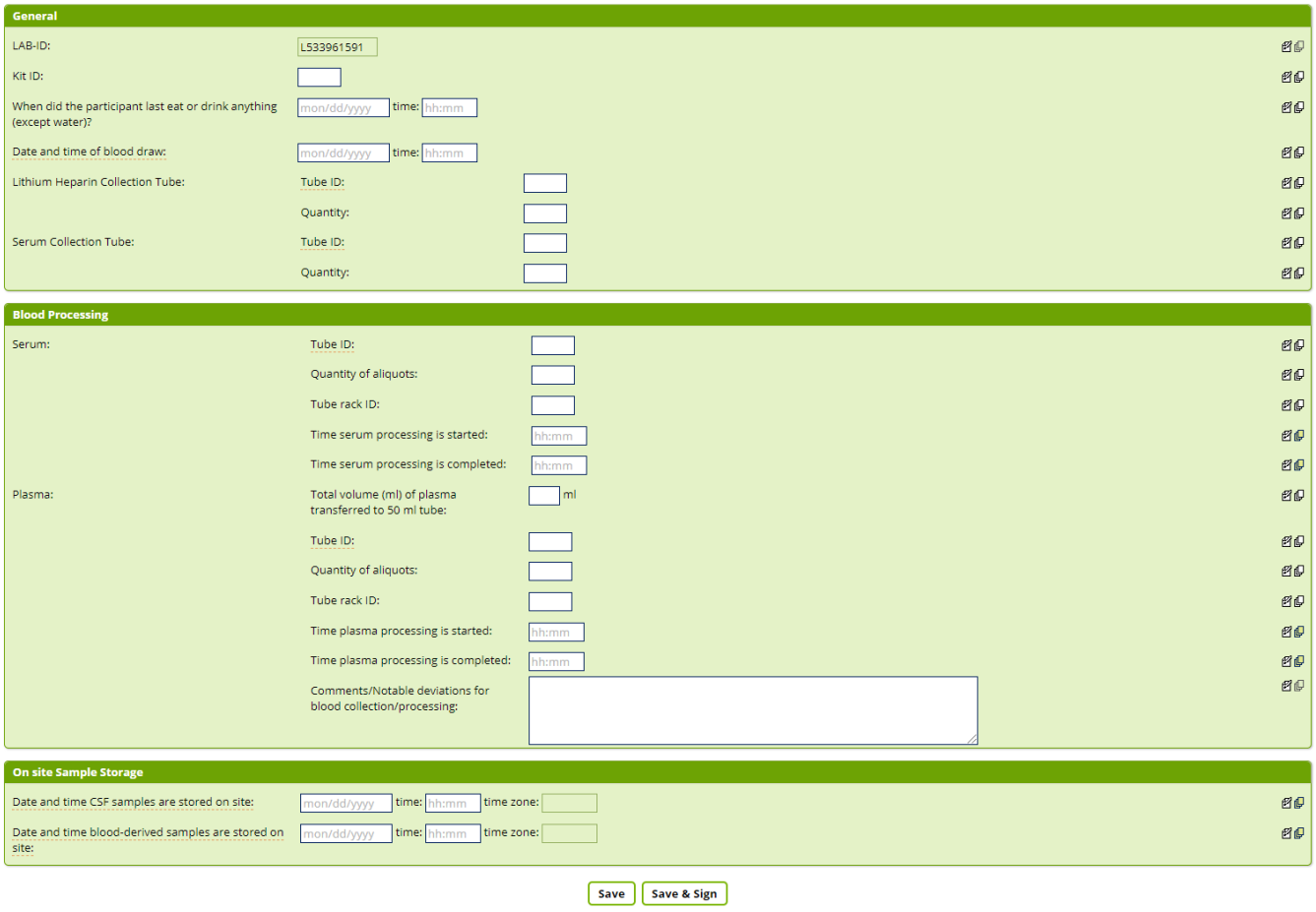
Screen shot 2: CSF



Screen shot 3: CSF Quality



Screen shot 4: Blood Processing



8 SHIPMENTS AND KIT ORDERING

8.1 SHIPMENT 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. One sample set consists of all samples (CSF, CSF cells, plasma, serum) from a single participant at a single sampling visit. If a participant has had more than one sampling visit (i.e. an Initial and Optional Repeat Sampling visit), two separate bags should be used. • Place one ***inactivated*** WarmMark temperature tag per sample set in the -80ºC freezer to await the scheduled shipment date • Ensure that there is 1 temperature tag for every sample set (the goal is to have a shipment of 4 participant samples sets, with 4 temperature tags per shipment, unless otherwise instructed by HDClarity CC)
2. Samples should be shipped to BioRep when you have 4 participant sample sets. The only exception to this is if you have reached the capacity of your freezer or if directed to ship samples by HDClarity CC, where 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 (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. Once you have contacted BioRep to schedule the collection, the courier will call/email you using the contact details you have provided. During this call/email please confirm the following:
    a. Quantity of participant sample sets you are shipping.
    b. Type and quantity of boxes you will need (a box size GDI 30 will accommodate 4 sample sets).[1]
    c. That 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.

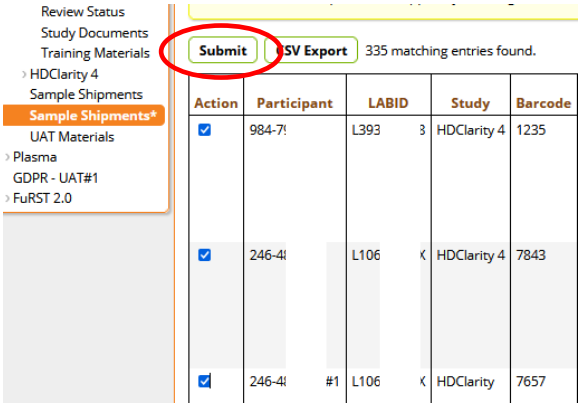
6. The courier may request a draft copy of the completed and signed 'No X-ray' form and/or the 'proforma invoice' before the collection so that they can ensure that the format and contents of the document will facilitate a smooth customs clearance.

    Note:** For sites using World Courier, please send World Courier the EDC generated proforma invoice and No X-Ray form at least 3 days ahead of the scheduled shipment. **In addition, couriers may refer to the proforma invoice as the "customs invoice".

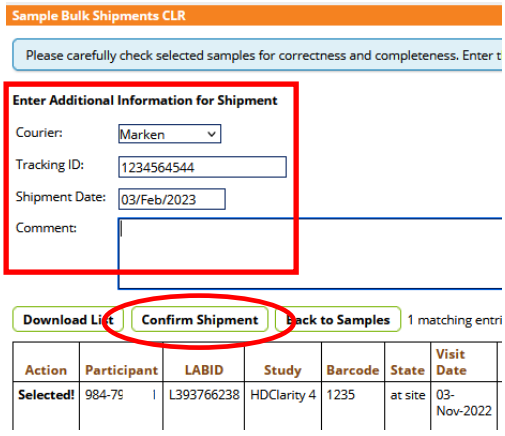
7. **If the courier has not contacted you at least 24 hours in advance of the shipment date, then please inform HDClarity CC immediately.**

8. 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.

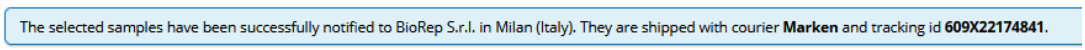
9. To notify BioRep of the scheduled shipment: Go to the Sample Shipment page on the EDC. Choose the filter “at site” and select all samples that shall be added to the shipment. Then click Submit.



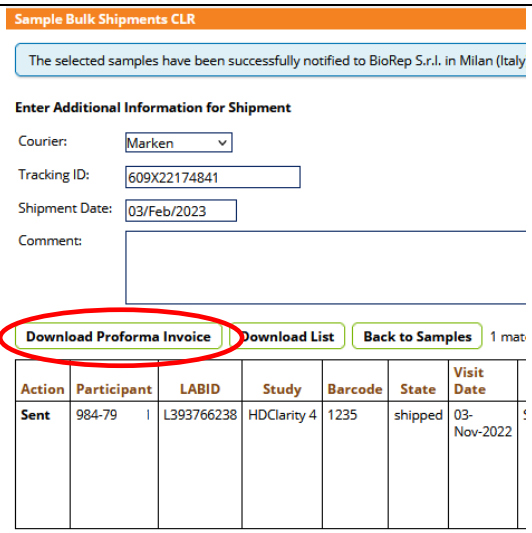
Select the Courier, enter the tracking number (World Courier or Marken AWB number) supplied by the courier, the shipment date and optionally you may enter a comment, if needed.
Confirm the entries with the ‘Confirm Shipment’ button. This action generates a notification to ensure that the biorepository is aware that the shipment will be in transit. (The 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).



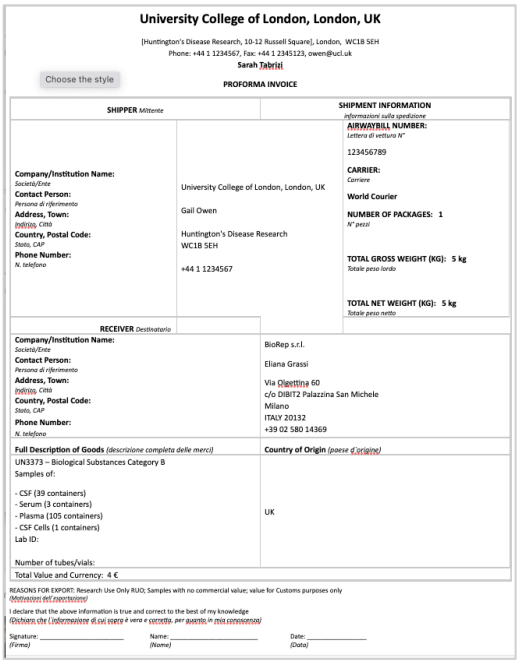
A successful submission is confirmed with a blue bar above the form:



For non-EU sites: Use the button ‘Download Proforma Invoice’ to create the necessary documents that shall accompany the shipment. *Note: EU sites do not need a Proforma Invoice but WILL need a No X-Ray form (see template on Appendix 2).



In the proforma invoice all necessary information is pre-entered[1]. It is important that you check the content. Missing items will be highlighted and will need to be added manually. Print 4 copies, date and sign them. Three copies of the proforma invoice will accompany the shipment, one shall be retained on site.



University College of London, London, UK

[Huntington's Disease Research, 10-12 Russell Square], London, WC1B 5EH
Phone: +44 1 1234567, Fax: +44 1 2345123, owen@ucl.uk
Sarah Tabrizi

Choose the style

PROFORMA INVOICE

SHIPPER *Mittente*		**SHIPMENT INFORMATION** *Informazioni sulla spedizione*
Company/Institution Name: *Società/Ente*	University College of London, London, UK	**AIRWAYBILL NUMBER:** *Lettera di vettura N°* 123456789
Contact Person: *Persona di riferimento*	Gail Owen	**CARRIER:** *Carriere* World Courier
Address, Town: *Indirizzo, Città*	Huntington's Disease Research WC1B 5EH	**NUMBER OF PACKAGES:** 1 *N° pezzi*
Country, Postal Code: *Stato, CAP*		**TOTAL GROSS WEIGHT (KG):** 5 kg *Totale peso lordo*
Phone Number: *N. telefono*	+44 1 1234567	**TOTAL NET WEIGHT (KG):** 5 kg *Totale peso netto*

RECEIVER *Destinatario*	
Company/Institution Name: *Società/Ente*	BioRep s.r.l.
Contact Person: *Persona di riferimento*	Eliana Grassi
Address, Town: *Indirizzo, Città*	Via Olgettina 60 c/o DIBIT2 Palazzina San Michele Milano
Country, Postal Code: *Stato, CAP*	ITALY 20132
Phone Number: *N. telefono*	+39 02 580 14369
Full Description of Goods *(descrizione completa delle merci)*	**Country of Origin** *(paese d'origine)*
UN3373 – Biological Substances Category B Samples of: - CSF (39 containers) - Serum (3 containers) - Plasma (105 containers) - CSF Cells (1 containers) Lab ID: Number of tubes/vials:	UK
Total Value and Currency: 4 €	

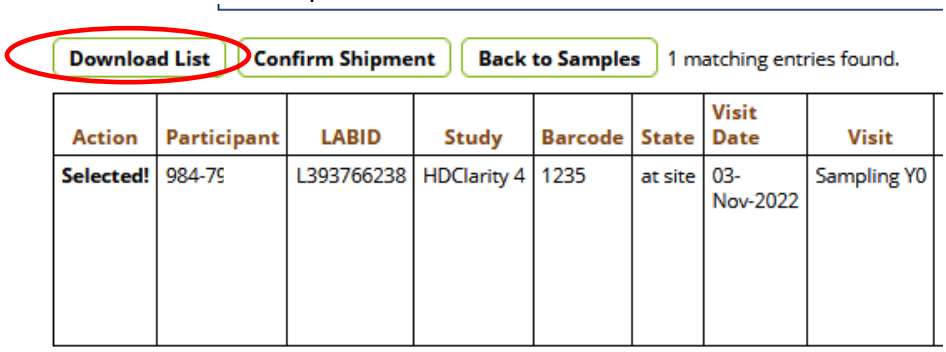
REASONS FOR EXPORT: Research Use Only RUO; Samples with no commercial value; value for Customs purposes only
(Motivazioni dell'esportazione)

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)*

The type of information included in the Proforma Invoice varies by sample type and by study. Furthermore, the information required by the Customs may change overtime. Therefore, the example of Proforma Invoice is for illustration purposes only and may not identically match the current implementation in the live EDC.

For all sites: Create a list of samples to be shipped from the EDC.
After the notification to Biorep, as seen above, click 'Download List' to generate a file with all selected samples.



An .rtf file opens (usually in Word). This list can be used to retrieve the correct samples for the shipment from the freezer.

Information on 4 Selected Samples:

- HDClarity 4 Sample W10080806 #1 labeled 1235 has state at site,
- HDClarity 4 Sample W10080907 #1 labeled 1235 has state at site,
- HDClarity 4 Sample W10081009 #1 labeled 1235 has state at site,
- HDClarity 4 Sample W10081108 #1 labeled 1235 has state at site,

10. Shipping documents
    All sites must complete and send the 'No X-ray' form provided to you by HDClarity CC (see 'Shipping Documents').

    Again, sites outside of the EU must send a 'proforma invoice' along with the shipment. The Proforma invoice should be generated via the EDC. If this is not possible, the form can be filled manually (see 'Shipping Documents').

Day of the shipment

11. When the courier arrives to collect the shipment:
    a. Use the sample list to retrieve the samples from the freezer.
    b. Take out the pre-cooled WarmMark temperature tags from the -80°C freezer, activate them by folding and pulling out the top tab completely, and place them in the document pouch of each biohazard bag containing one participant sample set.
    c. Check that the World Courier or Marken AWB number you received over

the phone matches the AWB number they have. d. Check the quantity of dry ice covers the samples. e. **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. You can use the EDC to track which shipments are at the site, shipped or arrived to BioRep and if there are any damaged or missing.
13. 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.
14. 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 4-participant box.

8.2 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 only after all sampling visit data is entered on the eCRF, all remote monitoring queries are resolved, and when you have at least 4 participant samples, or are 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 shipments 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.

8.3 PACKAGING PROCEDURES

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

Place the following into the Biohazard bag provided, per participant:

1. Rack containing all blue-topped cryovials labeled ‘CSF’ and single yellow-topped cryovial labeled ‘Cell CSF’

2. Rack containing all red-topped cryovials labeled ‘Plasma’

3. Rack containing all white-topped cryovials labeled ‘Serum’

To seal the biohazard bag, remove the white plastic from the top of the bag, revealing the adhesive strip, and fold it over. Press the seal firmly to ensure proper closure.

Place one inactivated WarmMark temperature tag in the document pouch of each sealed biohazard bag (four total, one for each participant sample set) and place in the -80°C freezer until shipment.

Picture depicts: Red plasma rack, blue CSF rack with one ‘Cell CSF’ yellow cryovial, white serum rack, and one inactivated WarmMark temperature tag inside the provided Biohazard bag, ready for shipment.

<u>To Be Completed on Day of Shipment:</u>

If using the WarmMark temperature tag, **make sure each temperature tag is activated.** Temperature tag activation requires the top edge of the tag (the indicator activation tab) to be folded over and pulled out completely (see photos below for examples).

Make sure there is one WarmMark temperature tag in each biohazard bag.

Inactivated Temp Tag:

Activated Temp Tag:

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.

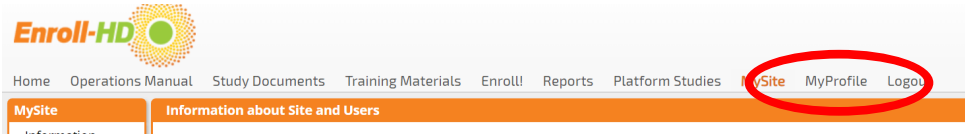
When the courier arrives, they will place the biohazard bags onto dry ice in the polyfoam shipping box. Please ensure the sealed biohazard bags with samples are fully covered with dry ice. Up to 4 bags may be shipped in any single shipment box (boxes and dry ice provided by World Courier or Marken).

8.4 SHIPPING DOCUMENTS

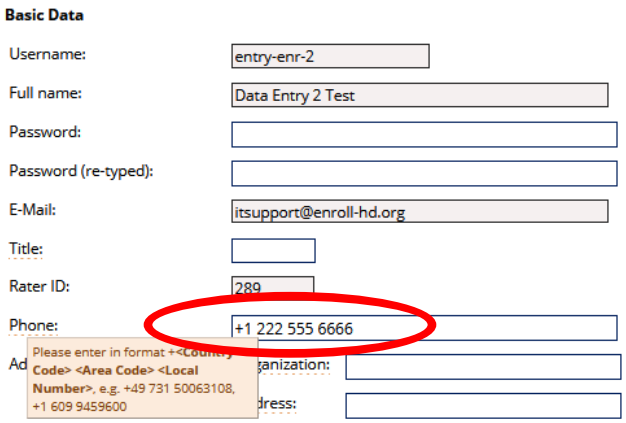
The courier will provide packaging and the prefilled AWB.

All sites must complete the 'No-X-ray' form (Appendix 2) 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 the EU must also complete the 'Proforma Invoice' form (Appendix 3) before the courier arrives to collect the samples. Please see EDC provided proforma invoices under section 8.1.9. Please check the content of the generated proforma invoice. Missing items will be highlighted and will need to be added manually. The proforma invoice tool will automatically pull all necessary information from the EDC, including the mandatory information name, email and phone number of the person creating the proforma invoice. To auto-populate the "Phone" field the contact person's phone number needs to be available in the "MyProfile" tab of the EDC.



Enter the phone number and click save.



If the site information used in the proforma invoice (i.e. address) is incorrect, please correct the respective proforma invoice manually and then contact your site support (i.e. site manager or lanco) to get the EDC information updated.

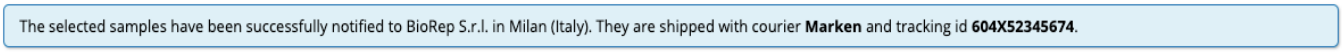
You must provide the courier with three copies of the form and these must be signed by the person at the site who has arranged the shipment. HDClarity CC will provide you with an electronic copy of this form and you must insert your site letter head to it. All sections highlighted must be completed.

! Please ensure Proforma Invoice includes KitID and/or LabIDs. KitID are also on the bags within the kit. No participant HDIDs 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.

*Remember to enter the World Courier or Marken AWB number of the bulk shipment container into the EDC and complete the shipment notification to ensure that the biorepository is aware that the shipment will be in transit.

A successful notification to the biorepository is shown like that in the EDC:



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

8.5 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. Your local supply should not drop below 5 kits unless recruitment has been paused. Or if recruitment is low, and you have fewer than 5 participant sampling visits planned, you will need sufficient kits for at least the number of planned sampling visits. If you expect your recruitment to be lower than 5 for ~3 months, reach out to HDClarity CC immediately to place an order for less than 5 kits outside of the EDC.

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

6. Any unused tubes or cryovials should be discarded by your site.

7. 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).

9 TRAINING DOCUMENTS AND 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 study documents. The repository location is: https://studies.enroll-hd.org/platform/clr/clr4/docs

There are three training videos for HDClarity. The first video demonstrates sample collection, the second shows sample processing, and the third demonstrates the use of the multipette and tips (if applicable to your site).

HDClarity training videos and documents are hosted on the Enroll-HD Clinical Training portal at: https://hdtraining.enroll-hd.org

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.

10 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.

11 CONTACT DETAILS

For enquiries then please use the following contacts:

hdclarity-cc@enroll-hd.org	HDClarity Central Coordination Team
hdclarity-physician@enroll-hd.org	HDClarity study physician (clinician)
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.

12 APPENDICES

Appendix 1 NEUROLOGICAL EXAMINATIONS

Full Neurological Exam

A full neurological examination and a brief physical examination is required during the Annual Screening Visit. Evidence of possible bleeding tendency such as bruises or petechial rash should be noted.

- Cranial nerves
  - visual acuity
  - visual fields to confrontation
  - fundoscopy (including appearance of discs and presence / absence of venous pulsations)
  - smooth pursuit and saccadic eye movements
  - facial sensation
  - jaw power
  - facial symmetry and power
  - bedside auditory acuity
  - palatal elevation
  - pharyngeal sensation
  - cough
  - Sternocleidomastoid muscle and trapezius power

- Upper and lower limbs
  - Tone
  - Proximal and distal power
  - Reflexes (-, +/-, +, ++, +++)
  - Pinprick sensation
  - Plantar responses
  - Coordination

Focused Neurological Exam

Each Annual Sampling Visit requires a focused neurological examination consisting of verbal confirmation of any changes compared to the previous full examination during the Annual Screening Visit. A brief physical exam is repeated for safety during the Annual Sampling Visit.

Appendix 2 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 3 - Proforma Invoice (non-EU sites, including UK as of January 1, 2021)

Please note, make use of the EDC provided, prefilled proforma invoice

>>Letterhead of your site<<

PROFORMA INVOICE

SHIPPER *Mittente*	**SHIPMENT INFORMATION** *informazioni sulla spedizione*	
Company/Institution Name: *Società/Ente* **Contact Person:** *Persona di riferimento* **Address, Town:** *Indirizo, Città* **Country, Postal Code:** *Stato, CAP* **Phone Number:** *N. telefono* **Email:**		**AIRWAYBILL NUMBER:** *Lettera di vettura N°* XXXXXXXXX **CARRIER:** *Carriere* **World Courier** **NUMBER OF PACKAGES: X** *N° pezzi* **TOTAL GROSS WEIGHT (KG): X** *Totale peso lordo* **TOTAL NET WEIGHT (KG): X** *Totale peso netto*
RECEIVER *Destinatario /* **IMPORTER OF RECORD**		
Company/Institution Name: *Società/Ente* **Contact Person:** *Persona di riferimento* **Address, Town:** *Indirizo, Città* **Country, Postal Code:** *Stato, CAP* **Phone Number:** *N. telefono* **VAT and EORI Number:**	BioRep s.r.l. Eliana Grassi Via Olgettina 60 c/o DIBIT2 Palazzina San Michele Milano ITALY 20132+39 02 580 14369 03891970968	
Country of Origin *(paese d`origine)*		

Full Description of Goods *(descrizione completa delle merci)*		
UN3373 – Biological Substances Category B. HS code 3002120090 - Human, CSF and serum. HS code 3002120010 - Human, plasma. Samples with no commercial value; value for Customs purposes only *(Motivazioni dell´esportazione)* 1 Kits: XXXX Containing XX Samples: CSF (XX vials), CSF Cells (X vials), Serum (X vials), Plasma (X vials) Total Volume of the Samples: X ml		
Total Value and Currency:	8.50 EUR	

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

REASONS FOR EXPORT *(Motivazioni dell'esportazione)*: Research Use Only RUO *(solo scopo di ricerca);* **INCOTERM CODE: DDP**

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: _____ *(Firma)*	Name: _____ *(Nome)*	Date: _____ *(Data)*

Appendix 4 - Biosample Destruction Request Form

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

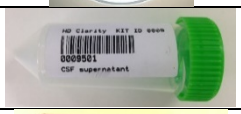
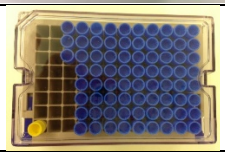
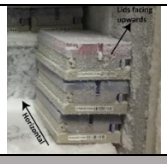
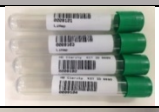
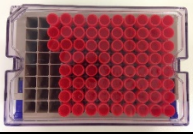
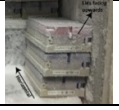
Stored Specimen(s) requested to be destroyed as a Stored Specimen at BioRep under the Services Agreement

Project's name: HDClarity	**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 ☐ Optional Repeat Sampling

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

Appendix 5 HDClarity Biosample Processing Summary

CSF PROCESSING		
Agitate the entire CSF sample for 10 seconds using a vortex mixer to homogenise the CSF. Then extract 200 µl of the CSF for cell counts.		Processing must start within 15 min and CSF cell counts within 60 min of sample collection. Use only provided polypropylene pipette tips.
Centrifuge the 50ml tube containing residual CSF at **400 × g for 10 min at 4°C, brake off.**		Balance the centrifuge and with clearly labelled water-filled balance tube.
Transfer the supernatant into the single green-topped 30ml polypropylene tube labelled 'CSF supernatant' and agitate for 10 seconds to homogenise the CSF.		Do not discard the blue-topped 50 ml CSF collection tube until the cell pellet has been extracted.
Aliquot the CSF in 300 µl aliquots into the pre-cooled cryovials with **blue** lids, labelled 'CSF', on wet ice.		CSF should be filled up to the printed black line on every cryovial. A 20ml CSF sample should produce at least 60 aliquots.
Re-suspend the CSF cell pellet in 300 µl of supplied RNA*later* solution and transfer the cell pellet solution to the cryovial with a yellow lid labelled 'Cell CSF'. Place cryovial in matrix rack with the other blue lidded CSF aliquots.		There should only be 1 'Cell CSF' yellow-topped cryovial. This is not the same as the 200 µl aliquot that is used for triplicate cell count.
Immediately after processing, place the CSF aliquots and the resuspended cells in your -80°C freezer within 5 minutes.		Ensure samples are stored upright and all lids are secure. Do not wait for blood processing to be completed.
BLOOD PROCESSING		
Spin the lithium heparin tubes at **1300×g for 10 min at 4°C, brake off** immediately on arrival.		Plasma processing must start within 15 minutes of sample collection.
Combine the supernatant in one tube labelled 'Plasma' and mix by inverting 10 times before placing on wet ice.		Discard any tubes whose plasma is pink due to haemolysis.
Aliquot the plasma in 300 µl aliquots into the pre-cooled cryovials with **red** lids, labelled 'Plasma'.		Plasma should be filled up to the printed black line on every cryovial. Four lithium heparin tubes should produce at least 35 aliquots.
Spin serum tube at **2000×g at room temperature for 10 minutes, brake off.**		The serum must sit un-spun at room temperature for 30 minutes (and no more than 40 minutes) to allow for clotting.
Transfer the supernatant into the single green-topped **15ml** polypropylene tube labelled 'Serum' and agitate for 10 seconds to homogenise the Serum.		
Aliquot the serum in 300 µl aliquots into the room temperature cryovials with white lids, labelled 'Serum'.		Serum should be filled up to the printed black line on every cryovial. One serum tube should produce at least 8 aliquots.
Immediately after processing, place the plasma and serum samples in your -80°C freezer within 5 minutes.		Ensure samples are stored upright and all lids are secure.