Plasma LiHep Collection for C-Peptide

SOP INN05

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2. Revision History

Version	Date	Description	Author
1.0	08/02/2017	New SOP	UCAM
2.0	26/0502017	Clarifications throughout	UCAM
3.0	03/06/2020	Sample shipment moved to SOP-INN09 Further clarifications and format change	UCAM
4.0	17/02/2022	Updated to include Patients at Increased Risk of Developing T1D	UCAM

3. Related Documentation

- Trial Specific Study Manual
- SOP INN08 Sample Storage and Transfer
- SOP INN09 Sample Shipment
- Sample Management User Guide
- Site specific SOPs/User Manuals i.e. centrifuge user manual; Instructions for Packing Samples for Dry Ice Shipment; freezer temperature monitoring; working in the lab etc

4. Important Notes

This standard operating procedure (SOP) must be read in conjunction with SOP INN08.

This SOP is for Unaffected Family Members (UFM) follow up visits (autoantibody positive participants), Patients at Increased Risk of Developing T1D (PIR) follow up visits and Newly Diagnosed (ND) baseline and follow up visits.

All samples originally collected and the aliquots generated from them must be logged into the INNODIA Data Warehouse https://innodia.cpr.ku.dk/login at collection / preparation point or, if this is not feasible, before freezing.

- **Gloves must be worn at all times when handing samples to avoid contamination with RNase, DNase, DNA and pyrogen.**
- **Sterile filter-tips must be RNase, DNase, DNA and pyrogen free.**
- **The samples must not undergo freeze-thaw cycles.**
- **Use one filter tip per each sample.**
- **At the end of centrifugation, the plasma must be aliquoted immediately.**

5. Materials

- Lithium Heparin tubes: 6 per OGTT; 7 per MMTT
- FluidX® tubes type A (orange caps) provided by INNODIA: 12 per OGTT, 14 per MMTT
- Insulated container with wet ice
- Centrifuge (preferably a cold-centrifuge, +4°C)
- Pipette with sterilized filter tips (RNase, DNase, DNA and pyrogen free)
- Freezer (-65°C or lower)

6. Sample Collection

- Collect blood into Lithium Heparin tube(s), invert gently 10 times and keep on wet ice.

Note: Blood tubes can be kept on wet ice for a maximum of 1 hour. If a bedside microfuge is used then samples do not need to be placed on ice if spun within 5 minutes of collection.

7. Sample Processing

- Centrifuge the blood sample for 10 minutes at 2500g. A +4°C centrifuge is preferable. Follow Table 1 for centrifuge timings if a bedside microfuge is NOT used.

Table 1. C –peptide (Li Hep) samples and advised centrifuge timings (when a bedside microfuge is NOT used)

Time (mins)	Drink (**OGTT**)	C-peptide (Li Hep) sample taken	**Centrifuge spins**
-20		✓	**Spin 1**
0	Start of ingestion	✓	
10	End of ingestion		
30		✓	
60		✓	**Spin 2**
90		✓	
120		✓	**Spin 3**

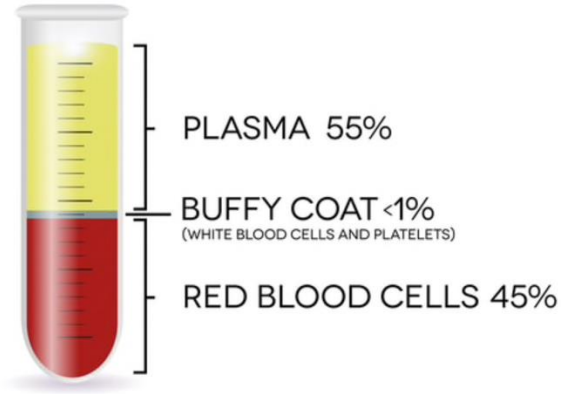
Time (mins)	Drink (**MMTT**)	C-peptide (Li Hep) sample taken	**Centrifuge spins**
-10		✓	**Spin 1**
0	Start of ingestion	✓	
10	End of ingestion		
15		✓	
30		✓	
60		✓	**Spin 2**
90		✓	
120		✓	**Spin 3**

- **IMMEDIATELY** after centrifugation, aliquot **2 x 0.3ml** plasma from each blood tube/timepoint into 2 x 0.5 ml FluidX® tubes type A (orange cap) provided by INNODIA.

Note: OGTT have **6** blood tubes/timepoints so there will be **12** aliquots in total.
MMTT have **7** blood tubes/timepoints so there will be **14** aliquots in total.

Note: It is very important not to pick up any red cells when aliquoting. This can be done by keeping the pipet 3-4mm above the buffy coat layer and leaving a small amount of plasma in the tube (Figure 1).

Figure 1. Buffy coat



Note: When splitting the parent sample between multiple aliquots, always ensure that the whole volume is used. If the volume is low, it is best to have fewer aliquots of the correct volume than multiple aliquots of a lower volume. i.e. if 2 x 0.3ml aliquots are required and there is not enough sample, aliquot 1 x 0.3ml rather than 2x 0.15ml.

- Scan the tube barcode and enter all requested information into the Data Warehouse at https://innodia.cpr.ku.dk/login.

- Place all aliquots upright on wet ice or transfer immediately to freezer (-65ºC or lower). All specimens should remain at -65ºC or lower prior to shipping. If a -65ºC or freezer is not available within 2 hours of preparation, storage in a -20ºC freezer or a -40ºC freezer is suitable, restrictions apply, see below and follow SOP INN008.

Note: Aliquots can remain on wet ice for a maximum of 2 hours before storing in a freezer (-65ºC or lower).

Note: If using a -20ºC freezer samples must be transported to a -65ºC or lower freezer at the end of the day as long as the -20ºC temperature can be maintained whilst the samples are being transported between the two freezers.

Note: If using a -40ºC freezer samples must be transported to a -65ºC or lower freezer within a month as long as the -20ºC temperature can be maintained whilst the samples are being transported between the two freezers.

Note: The samples must not undergo freeze-thaw cycles. This can happen when placing newly aliquoted samples on to an already partially populated rack. To avoid freeze thawing in this instance please ensure the rack is not removed from the freezer. If this is not possible please ensure the temperature remains but placing rack on dry ice. If freeze-thaw occurs, follow SOP INN08.

For Sample shipping, refer to study specific Study Manual and SOP INN09 for details and timeframes.