K3-EDTA Plasma Collection for Micro-RNA

SOP INN04

1. Table of Contents

Contents

2. Revision History

Version	Date	Description	Author
1.0	08/02/2017	New SOP	UCAM
2.0	26/05/2017	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.

General important background to procedures:

MicroRNAs (or miRNAs) are non-coding endogenous highly conserved 22 nucleotides long RNA sequences that regulate gene expression.
Blood has always presented a unique challenge to RNA isolation technologies. Many of the cells in blood are poised to rapidly respond to changes in their environment and, as a result, show changes in gene expression almost immediately after sample collection. During blood separation it is imperative that cell debris is removed.

It has been shown that RNA concentrations in plasma that were subjected to a single free-thaw cycle in comparison to frozen never-thawed plasma controls were significantly lower and concentrations declined with each additional plasma free-thaw cycle, therefore plasma must not undergo freeze-thaw cycles (Ge Q, *et al*, 2014).

Reference: Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z, *Molecules*, 2014, miRNA in plasma exosome is stable under different storage conditions.

- **Gloves must be worn at all times when handing samples to avoid contamination with RNase, DNase etc.**
- **All pipettes, pipette filter-tips, benches and centrifuges must be RNase, DNase, DNA and pyrogen free.**
- **Samples must not undergo freeze-thaw cycles.**
- **It is important for micro-RNA that the sample is not hemolysed, icteric or lipemic, and that it is processed maximal 2 hours from collection.**

5. Materials

- 1 x K3-EDTA tube
- 1 x tube for second centrifugation (RNase, DNase, DNA and pyrogen free)
- 5 x FluidX® tube type A (orange cap) provided by INNODIA
- Centrifuges set at room temperature (1800g) and 10 °C (1200g)
- Pipette with 200µl filter-tips (RNase, DNase, DNA and pyrogen free)
- Small ice bucket
- Freezer (-65°C or lower)

6. Sample Collection

- Collect blood into a K3-EDTA tube (2.6ml).
- **Invert 10 times** the K3-EDTA tube containing the blood collection.
- Store tube upright at room temperature for a **maximum of 2 hours**.

7. Sample Processing

- Centrifuge the blood sample for 10 minutes at 1800g at room temperature.

Note: Time, speed and temperature are important to avoid activation of micro-RNAs.

- Check plasma and note state, as shown in Figure 1, in the Data Warehouse (https://innodia.cpr.ku.dk/login). Do not discard if the plasma is hemolysed, icteric or lipemic as it can be used for other analysis.
- Transfer **plasma layer only** into a RNase, DNase, DNA and pyrogen free tube (Epperdorf style) for second centrifugation.

Note: Plasma should be transferred in a way that possible contamination is avoided. Please wear lab coats and gloves, effectively clean the work bench before and after with an appropriate cleaner. Please use sterilized filter tips (RNase, DNase, DNA and pyrogen free) ensuring the use of clean pipettes and change the pipette tip after each sample.

Note: Be very careful not to pick up blood cells when aliquoting. This can be done by keeping the pipet 3-4mm above the buffy coat layer (figure 1) and leaving a small amount of plasma in the tube.

- Centrifuge the plasma sample for 20 minutes at 1200g at 10°C to remove cell debris.

Note: Time, speed and temperature are important to avoid activation of micro-RNAs.

- Aliquot 0.2ml of plasma supernatant into each of five FluidX® type A tube provided by INNODIA. Close the caps on the vials tightly.

  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 5 x 0.2ml aliquots are required and there is not enough sample, aliquot 4 x 0.3ml rather than 1 x 0.15ml.

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

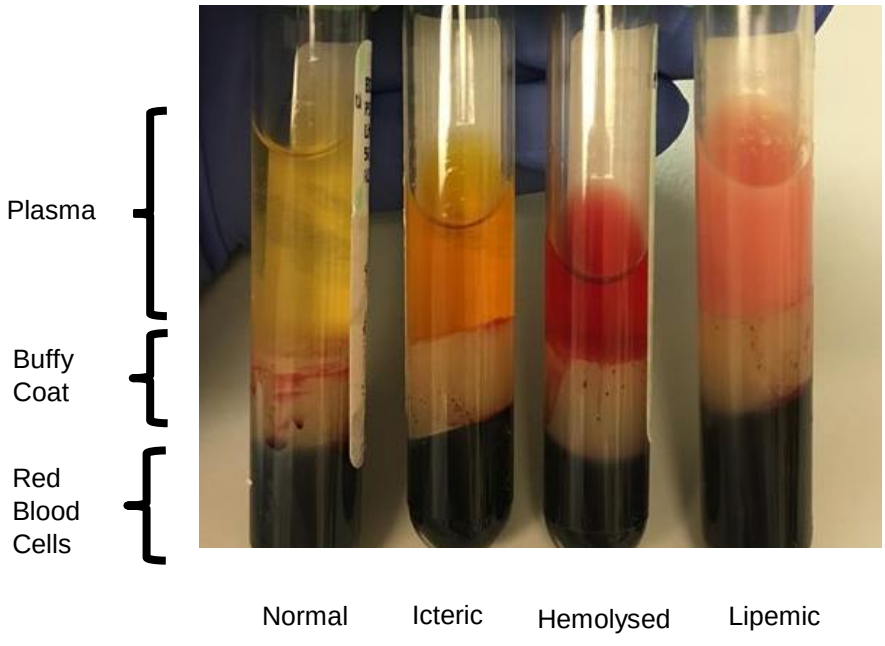
- Place all aliquots upright in a small ice-bucket and transfer to freezer (-65ºC or below). All specimens should remain at -65ºC or below prior to shipping. If a -65ºC freezer is not available storage in a -20ºC freezer or a -40ºC freezer is suitable, restrictions apply, see below and follow SOP INN008.

Note: If using a -20ºC freezer samples must be transported to a -65ºC 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 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 by placing rack on dry ice. If freeze-thaw occurs, follow SOP INN08 and record on the Data Warehouse at https://innodia.cpr.ku.dk/login .

Figure 1 Analysis of plasma after centrifugation step. Layers shown (left, from top down) are plasma, buffy coat and red blood cells. Plasma layer must be normal (left) to be used in micro-RNA analyses. Sample cannot be used if plasma layer is hemolysed, icteric or lipemic.



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