DAISY Clinic Visit: Blood Sample Processing

Materials	Cryovial Tray Etched Cryovials: 2ml, 0.5ml, 0.5ml Amber, 2ml Amber Cryomarkers Transfer Pipettes Yellow Pipette Tips Blue Pipette Tips Adjustable Volume Pipettes Sample Storage boxes Blood Sample collected in Red Top (serum separator tube) Blood Sample collected in foil-wrapped Purple Top (plasma tube) Blood Sample collected in foil-wrapped Green Top (plasma tube) for IVY visits Blood Sample collected in ABI mRNA tube Blood Sample collected in grey microvette tube for DM positive visits Urine in Specimen cup for DM positive visits Clinic Visit and Sample Tracking form Daisy labeling map Daisy Tracking folder Daisy Logbook Purple Top Plasma Distribution table Hemostats
Procedure	1. When subject arrives label Cryovials with Cryomarkers according to the Daisy labeling map and create a sample grid in the logbook (found in the lab file basket.) 2. If subject is DM AB+ take and A1c cartridge out of the refrigerator and place it next to the HbA1c machine. 3. After Blood samples are received from Clinic staff, allow Red Top sample to completely clot (10-30 minutes), write time samples were dropped off in the lab on the sample rack. 4. Place all foil-wrapped tubes in the refrigerator until Red Tops are ready to spin. 5. Run the HbA1c from the grey top (if subject is DM AB+), or unwrapped Purple Top tube if grey top has not been provided. See **DCA 2000 Sample Analysis** procedure. 6. Once A1c value has been obtained, grey top can be discarded unless HemoCue value is needed • If random glucose >100 for a fasting subject Or If random glucose > 140 for a non-fasting subject – Go To **HemoCue Sample Preparation and Analysis Procedure** 7. Assign a LabID and place stickers onto the lower right corner of the Clinic Visit and Sample Tracking form and LabID tube. 8. If ABI mRNA tube is collected, make sure there is pink tape on the tube just below the cap, and check for subject ID and draw date on the tube, assign this sample a spot via the mRNA grid found in the tracking folder and write this location on the pink tape on the tube and on the Clinic Visit and Sample Tracking form. 9. Shake ABI mRNA tube vigorously for 20 seconds-keep at room temperature for minimum 2 hours (sample can be left at room temp overnight if the last visit of the day and it can be stored in the a.m.) 10. Process samples as quickly as possible – Daisy samples are higher priority than Teddy visits due to the temperature and light sensitivity of the vitamin (8s, 9s and 10s) samples.

	11. Check the Clinic Visit and Sample Tracking form to determine if whole blood needs to be collected for the subject. See **Whole Blood Sample Processing** procedure. 12. Spin blood samples according to manufacturer's recommendations: 13. Red Top, Purple Top and Green Top; 10 minutes at 3000 rpm (may be spun with CPT tube at 4000 rpm) 14. **Do Not** Spin ABI mRNA tube. 15. While blood is spinning transfer box numbers from the grid book to the Clinic Visit and Sample Tracking form. 16. Urine samples can be aliquotted at this time using the volumes listed on the DAISY labeling grid. 17. After spinning, process samples from the foil-wrapped tubes first as they are heat and light sensitive. 18. Check the Clinic Visit and Sample Tracking form to determine if DNA needs to be collected for the subject. See **DNA to BDC Processing** procedure. Check clinic visit form to determine if fasting insulin sample needs to be collected. If present, see Fasting Insulin Processing procedure. 19. Make a hole in the foil of the foil-wrapped Purple Top tube immediately before aliquoting from it using hemostats. Be sure that the hole is large enough that the plasma, buffy coat, and red blood cell layers can be seen to ensure the removal of the correct sample type. Use the plasma from this tube only for amber cryovials, the unwrapped purple top will be used for the clear cryovials 20. Using the adjustable volume and transfer pipettes, and beginning with the foil Purple Top tube, transfer 500µL of plasma into each 8s.(2 8's tubes) 21. Transfer 1500µL into the first two 10s Cryovials and split the total remaining volume into the 2s Cryovials. • If needed, up to four additional 2s Cryovials can be created to hold excess storage. • Fill as many 2s Cryovials with 1000µL as possible. If needed, the 2s Cryovials can be filled to a maximum volume of 1500µL, do not discard any plasma! 22. Remove the Buffy Coat from one of the Purple Top tubes and place it in the 3 1 Cryovial, discard this Purple Top tube. 23. Write volumes for each of the storage Cryovials on the Clinic Visit and Sample Tracking form (10s,and 2s.) 24. Make a hole in the foil of the foil-wrapped Green Top tube (if the subject is an IVY participant) immediately before aliquoting from it using hemostats. Be sure that the hole is large enough that the plasma, buffy coat, and red blood cell layers can be seen to ensure the removal of the correct sample type. 25. Using the Green Top tube (if the subject is an Ivy participant) and the adjustable volume pipettes aliquot plasma into the 9 1, and 9 2, Cryovials using the volumes listed on the DAISY labeling grid. Transfer the remaining plasma into 9 3 using a transfer pipette, then remove and discard the buffy coat. 26. Transfer all of the red blood cells from the Green Top tube into the RBC Cryovials (500µL in the first Cryovial, and the remainder in the second.) 27. Write volumes for each of the storage Cryovials on the Clinic Visit and Sample Tracking form (9 3 and RBCs.) 28. Transfer the 9s, RBCs, and 10s Cryovials into the liquid nitrogen, be sure to place a t-shirt piece in the liquid nitrogen tube so that the Cryovials are not lost in the liquid nitrogen. See **DAISY Processing**

	FAQ if tubes are lost in the liquid nitrogen. 29. Using the Red Top tubes and a transfer pipette, fill the LabID Cryovial first with 1000µL of serum, then fill as many 1s Cryovials with 1000µL as possible. If needed, the 1s Cryovials can be filled to a maximum volume of 1500µL, do not discard any serum! 30. From one of the 1s tubes, aliquot volumes for the QC Cryovials using the adjustable volume pipettes and the volumes listed on the Daisy labeling map. Please note that LabID and QC Cryovials are the highest priority. 31. Write volumes for each of the storage Cryovials on the Clinic Visit and Sample Tracking form (1s) and list the number of tubes collected for each sample type along the left side of the page. 32. Remove the 9s, RBCs, and 10s Cryovials from the liquid nitrogen and put them back in the sample tray. 33. Using the sample storage boxes found on the Daisy freezer shelf put each sample Cryovial type away, double check the box numbers on the Clinic Visit and Sample Tracking form against boxes in the freezer and write the box space number for each Cryovial on the Clinic Visit and Sample Tracking form (a space number is not required for the LabID sample.) 34. Fill out the grid with the number of Cryovials of each sample type placed in the freezer and place a “T” next to the grid. Underline the first 1s sample listed on the grid to indicate that it will be sent out for antibody testing.