

DAISY LAB

B. Processing Samples

Set-up

Viral cultures are ready for immediate storage in the freezer, but the rest of the specimens need to be processed.

Specimens are stored in either 2ml or 0.5ml crotubes. IVY samples are stored in amber tubes to protect them from light, except for RBCs. Each cryotube stored is labeled with the DAISY ID, date, type of specimen, and tube number. The following codes are used for type of specimen:

Q: QCs (Serum)

1: Serum

2: Plasma (non-IVY)

3: Buffy coat

4: Saliva

5: Viral cultures

6: Whole blood

7: Urine

8: IVY plasma (purple top, CRC)

9: IVY plasma (green top, Deutsch)

10: IVY plasma (purple top, Awad)

11. RBC: Red blood cells

12. ABI tube (mRNA)

Some samples are not stored by DAISY, but are sent to the BDC for testing for diabetes antibodies and/or TGIgA. These samples are bar-coded. The barcode is a unique identifier for subject and date of visit. Sets of stickers with barcodes on them are provided in the lab. A sticker is placed on each tube to be sent to the BDC: diabetes antibody cryotube, TG cryotube, and BDC DNA purple top. A sticker is also placed on the clinic visit sheet for tracking. The remaining stickers are placed on the tracking sheets. The tracking sheets are used to keep a record of which subjects have had samples sent to the BDC in a particular week. There are tracking sheet for diabetes antibodies and TG. Along with the sticker, the DAISY ID and date are recorded on the tracking sheet. If a BDC DNA purple top tube is collected, the date, barcode ID, and DAISY ID are written on a different tracking sheet. If we are seeing multiple family members, the barcodes should be coded in order of family id, i.e. 00997-0 gets the first barcode, 00997-1 gets the second, 00997-2 gets the third, 00998-0 gets the fourth, etc.

DO NOT BARCODE ANYTHING UNTIL YOU HAVE ACTUALLY RECEIVED THE BLOOD!

Specimen Aliquots

IF SUBJECT IS IVY, IVY BLOOD SHOULD BE PROCESSED FIRST!

All samples are aliquotted directly from the vacutainer or specimen cup into a 2ml cryotube using a bulb pipet. Some samples are then aliquotted in specific amounts from the 2ml cryotube into a 0.5ml cryotube using the adjustable volume pipets. Here are the volumes to aliquot after centrifuging blood:

IVY Green top: Plasma is pipetted into 2ml amber tube. Buffy coat is discarded and RBCs are pipetted into 2ml tube. Plasma is aliquotted into 3 0.5 ml amber tubes: 60ul, 100ul, 200ul. The amount remaining in the 2ml tube is variable, but should be recorded.

IVY Purple top: Revised plasma aliquot schedule-see table in lab. 1.5ml of plasma is pipetted into 2ml amber tube, and repeated. A 0.5ml plasma aliquot is collected from each 2ml tube into 2 0.5ml amber tubes. Any remaining plasma is pipetted into 2ml tube for DAISY storage. The amount is variable but should be recorded. Up to 5 tubes of plasma may be stored. Buffy coat is collected in a 2ml tube. Only one tube of buffy coat is stored. If the subject is not IVY, all plasma in the purple top is collected for DAISY storage, and buffy coat is also collected in a 2ml tube.

AFTER IVY BLOOD IS ALIQUOTTED, IT IS IMMEDIATELY PLACED IN LIQUID NITROGEN FOR SNAP FREEZING.

Serum: From the red top, 1.0 ml is pipetted into the labid labeled 2ml tube. This tube is sent to the BDC for diabetes antibody and TG antibody testing. The rest of the serum is pipetted into the 2ml tubes for DAISY storage. From these tubes, serum is aliquotted for the 0.5 ml TG and QC tubes. Three QCs are collected, 2 of which contain 0.2ml, and one with 0.1ml. For people who have tested positive for ANY antibody, and additional 0.2ml QC is collected. If TG testing is not being done, it is only necessary to collect 2 QCs. Any remaining serum is collected in 2ml tubes for DAISY storage. Up to 5 tubes may be stored.

Saliva: Saliva is pipetted from specimen cup into 2ml cryotubes. The amount in each tube is variable, but should be recorded. Up to 3 tubes of saliva may be stored.

Urine: Urine is pipetted from specimen cup into one 2ml cryotube. From the 2ml tube, three 1.0ml aliquots are collected into three 2ml cryotubes. The amount remaining in the last 2ml tube is variable, but should be recorded.

Special circumstances

Less than optimal amount of blood: Ideally, the phlebotomist will draw 20-30ccs of blood for each subject. This is more than enough to collect all the samples needed. Sometimes, due to low body mass of babies, small veins, or quickly clotting blood, it is not possible to get this much blood. If this happens, the red top DAISY tube is the highest priority, followed by the IVY tubes if the subject is in IVY. There are 'cheat sheets' that tell you how to divide the blood if less than 20ccs are obtained (Appendix **). These are in the lab and at each drawing station. Additionally, in the lab are instructions on how to divide the IVY plasma from the IVY purple top tube if less than 6ccs are obtained.

Filter paper: Sometimes it is impossible to get venous blood at all, or in an amount too small to put in the vacutainer. When this happens, the blood is put onto filter paper. We are

able to test for diabetes antibodies using filter paper. When putting blood onto filter paper, it is best to fill an entire circle before beginning a new circle. The filter paper is placed on weighing paper and left at room temperature for 24 hours, then filed alphabetically by last name in the -20 freezer. The clinic visit sheet is either given to Iman or left on the lab counter for Iman to pick up.

Subjects who do not consent to storage: For those subjects who do not consent to indefinite storage by DAISY (second check-box on the consent form), do not collect whole blood or buffy coat for DAISY storage. However, if it is the first clinic visit and we need their HLA type, collect whole blood for Roche as well as the extra 1.25ml of whole blood. This is collected in case we need to resend the blood for Roche. It is placed in the box in the fridge labeled 'Subjects not consenting to storage'.