DAISY LAB

B. Processing Samples

Take Blood to Laboratory for Processing

- Place any purple or green top tubes inside refrigerator
- Place red top tubes in upright position at room temperature to facilitate clotting process
  - To further accelerate clotting process, invert the tube several times immediately after blood is placed in tube
- When there are 2 purple top tubes collected, send one to the BDC for DNA if needed.
  - Do NOT spin the blood.
  - Keep it in the refrigerator until it is taken over to BDC.
  - Label the tube with the DAISY ID, the date collected, and also put the bar code (lab ID number) from that visit on it
  - Write the date collected, lab ID, and DAISY ID on the log sheet that is in the Ab's folder.
  - When entering the chart in the database, in the Samples Sent window, it is the DNA/BDC choice of blood and it is only sent for HLA (mark all others No)
  - Put all tubes collected in a ziplock bag marked with DAISY DNA name at the top and DAISY and the date at the bottom, and take them to DNA/HLA lab on the 4th floor of the BDC.
    - When you take them over, write the date you take them and your initials on the log sheet.
    - Put them in the refrigerator that is at the end of the first row of the lab (directly in front of you when you walk in the door of the lab). There is a cup marked DAISY in the door of the fridge to place them in.

<u>Aliquot Whole Blood</u>

- Whole blood must be aliquotted before centrifuging! (Refer to labeling and aliquotting protocol)

Centrifuge Blood

- After blood has clotted, tubes are ready for centrifuging
  - Place each tube in centrifuge, with a balance tube placed opposite of each
    - Balance tube must be the same type of tube as the sample tube containing blood (i.e. purple top, green top, red top)
    - Balance tube must contain a volume of water equal to the volume of blood in the sample tube
- Set centrifuge speed at 2, and centrifuge timer for 10 minutes
- After tubes have spun for approximately 30 seconds, increase speed to 5
- After tubes have spun for approximately 60 seconds, increase speed to 8
- Timer will automatically turn off after 10 minutes
- Centrifuge may take up to 2 minutes to stop spinning after timer turns off

Label Tubes and Aliquot Blood

- Place tubes in tube tray
- Tube layout and labeling:

ROW 1A: SERUM (red top tube)

200µL QC	LABELED ID#, DATE, QC
1000µL BDC/ TGIgA	LABELED BAR CODE
100µL TGIgA QC	LABELED ID#, DATE, TG
200µL QC2	LABELED ID#, DATE, QC2
200µL QC3	LABELED ID#, DATE, QC3 (Only if the subject is Ab positive)

ROW 1B: SERUM (red top tube)

1ml SERUM*	LABELED ID#, DATE, 1 1
1ml SERUM*	LABELED ID#, DATE, 1 2
1ml SERUM*	LABELED ID#, DATE, 1 3

*these volumes may vary *maximum of 7 tubes

From red top tube, aliquot 1000µL into BDC tube, and the rest into tubes in row 1B. From these tubes, pipet 100µL/200µL at a time into the remaining QC tubes.

ROW 8/10: CRC/AWAD (IVY only) (foil-covered purple top tube)

1ml PLASMA	LABELED ID#, DATE, 10 1
1ml PLASMA	LABELED ID#, DATE, 10 2
500µL PLASMA	LABELED ID#, DATE, 8 1
500µL PLASMA	LABELED ID#, DATE, 8 2

*Aliquot .5ml into the 8 1 and 8 2 tubes, with pipetman, then aliquot 1ml into 10 1 and 10 2 tubes.

ROW 9: DEU (IVY only) (foil-covered green top tube)

60µL PLASMA	LABELED ID#, DATE, 9 1
100µL PLASMA	LABELED ID#, DATE, 9 2
200µL PLASMA	LABELED ID#, DATE, 9 3
1ml PLASMA*	LABELED ID#, DATE, 9 4
500µL RED BLOOD CELL*	LABELED ID#, DATE, RBC 1
500µL RED BLOOD CELL*	LABELED ID#, DATE, RBC 2

*at least 500ul in RBC 1, if Vol_t <1000ul, remainder in RBC 2-if Vol_t >1000ul, split volume between RBC 1 & RBC 2 [volume change implemented 6/11/2010]

Aliquot all into last tube (9 4) then aliquot each "9" sample from 9 4, with pipetman, into each of the three other tubes. [volume changes implemented 10/16/2007]

ROW 2: PLASMA

1ml PLASMA*	LABELED ID#, DATE, 2 1
1ml PLASMA*	LABELED ID#, DATE, 2 2
1ml PLASMA*	LABELED ID#, DATE, 2 3

*these volumes may vary *maximum of 5 tubes

Aliquot plasma from purple top tube into labeled tubes. If subject is IVY, any plasma leftover from the foil-covered purple top goes into these tubes.

ROW 3: BUFFY COAT

500µL B.C.* LABELED ID#, DATE, 3 1
*volume may vary *only 1 tube of buffy coat is collected
After plasma is aliquotted from purple top, aliquot the buffy coat (white layer between plasma and red blood cells)

ROW 4: SALIVA-discontinued

1ml SALIVA* LABELED ID#, DATE, 4 1
1ml SALIVA* LABELED ID#, DATE, 4 2
1ml SALIVA* LABELED ID#, DATE, 4 3
*these volumes may vary *maximum of 3 tubes
Aliquot saliva from specimen cup directly into tubes

ROW 6: WHOLE BLOOD

500µL W.B. LABELED ID#, INITIALS
1250µL W.B. LABELED ID#, DATE, 6 1

*A 1250µL tube of W.B. is only stored on individual's first visit in the clinic. (Parents, sibs & all new study participants). If they have not had their gene typing done, the second tube (volume 500µL) is put in the Roche box to be sent for typing (parents, sibs & new SOCs).
Aliquot whole blood before centrifuging!

ROW 7: URINE-discontinued if Ab-Neg

500µL URINE into CLEAR CONICAL TUBE **LABELED 7 1**
500µL URINE into CLEAR CONICAL TUBE **LABELED 7 2**
250µL URINE into CLEAR CONICAL TUBE **LABELED 7 3**
1ml +/- URINE into CLEAR GRADUATED TUBE **LABELED 7 4**
*Aliquot first three with pipetman and then aliquot up to 1.5ml into 7 4.

- Labeling tubes with barcodes:
  - In blue lab folder, use one row of 6 barcodes for barcoding tubes
    - 2 barcodes are placed on the 2 barcoded tubes
    - 2 barcodes are used for recording samples sent to BDC for testing
      - Paper sheets for recording DM Ab's and TGIgA samples sent to BDC are found in blue folder
      - Record date and study subject's ID next to designated barcode
      - For samples sent for IgA testing, you must write barcode number down, along with date and subject ID
    - Barcode is placed on clinic sheet
    - Remaining barcode discarded or X'ed out
      - If extra purple top tube is obtained for DNA testing at BDC, the remaining barcode will be used to label this tube

- **Aliquot Priority**

- Aliquot IVY tubes first (IVY blood is sensitive to heat and light)
    - After aliquotting IVY blood into amber tubes, tightly cap tubes and snap-freeze in liquid nitrogen
        - Take a cylinder out of the nitrogen tank and drop tubes inside cylinder
        - Slowly lower cylinder into nitrogen tank
        - Leave tubes and cylinder inside tank for minimum of 30 seconds

    *****Use extreme care when working with liquid nitrogen tank! Liquid nitrogen can burn your skin!**
    - Finish aliquotting remaining blood/saliva/urine
    - Ensure that all tubes are capped tightly and labeled

Store Samples

- Current clinic sample storage boxes are kept on middle shelf of -70 lab freezer
- Put samples away in their respective boxes
  1-Serum
  2-Plasma (Non-IVY)
  3-Buffy coat
  4-Saliva
  5-Viral
  6-Whole blood—this is stored in lab refrigerator until box is full
  7-Urine
  8-IVY plasma
  9-IVY plasma
  10-IVY plasma
  11-RBC
    - When box is full, take box to basement freezer storage room and put it the designated area for the type of sample being stored
- While putting samples in storage boxes, record box and space numbers on clinic visit sheet
- Record number of tubes and their respective storage boxes in lab book, along with subject ID, name, and date

Clean Up Area

- After samples have been stowed in freezer, place tube tray in 10:1 water/bleach solution for decontamination
- Ensure that all pipets, specimen cups, vacutainers, and unused specimens are properly disposed of
- Clean any spills with alcohol wipes
- If necessary, replace diaper pad