Biospecimen Protocols

Section 1: Maternal Plasma, RBC, & WBC Processing

1. Centrifuge 10ml EDTA (purple top) vacutainer and 10ml Sodium Heparin (green top) @ 2000rpm, 4˚C for 10 minutes.

2. With a transfer pipette, remove plasma from purple top tube without disturbing the buffy coat into 2 NUNC tubes labeled PT=Purple top plasma.

3. Pipette white cell layer from purple top and transfer to 50 ml tube containing 15ml RBC Lysis Solution.
4. Shake 50ml tube to mix thoroughly then incubate on ice for 15 minutes.

5. Add one pipette full of saline solution to the remaining red blood cells in purple top tube and roll tube slowly to “fold in” saline solution.

6. For green top tube, transfer the plasma into NUNC tubes labeled GP=Green top plasma. Remove buffy white cell layer and discard.

7. Add one pipette full of saline solution to green top tube and roll tube slowly to “fold in” saline solution.

8. Centrifuge purple top and green top tubes at 2000rpm, 4º for 10 minutes.

9. Remove saline with transfer pipette; be careful not to disturb the red blood cells.

10. Add another pipette full of saline solution to blood tubes. Gently mix by rolling tubes and centrifuge at 2000rpm for 10 minutes.

11. Thoroughly remove the remaining saline solution after the second wash.

12. Aliquot the Red Blood Cells equally into NUNC tubes labeled PR=purple tube red blood cells. For Green top tube, aliquot the remaining Red blood cells into NUNC tubes labeled GR=green top tube red blood cells.

13. Spin 50 ml tubes containing White Blood Cells at 2000rpm, 4˚C for 10 minutes.

14. Pour supernatant into biohazard container.

15. Using the pipette man, aspirate 5ml of RBC Lysis Solution and re-suspend the WBC pellet by gently swishing the solution with pipette or thoroughly shaking. Place on ice to incubate for 5 minutes.

16. Spin at 2000rpm, 4˚C for 10 minutes.

17. Pour supernatant into biohazard container.

18. Remove remaining supernatant with a pipette, taking care not to disrupt the WBC pellet.

19. Re-suspend pellets in 1200ul NE Buffer, gently swish the solution with pipette until WBC pellet is evenly dispersed into the solution.

20. Transfer WBC solution to the properly labeled NUNC tube.

Section 2: PURAGENE® DNA Purification Protocol (Modified)

DNA Purification From Buffy Coat Prepared
From 10ml Whole Blood
Expected Yield Range 200-400 µg DNA Cell Lysis
Protocol courtesy of Tram Anh Tran (5/27/2014)

1. Dispense 10 ml of Cell Lysis Solution & RNase[1] into clean 50 ml tubes

2. Add WBC pellets to the 50 ml tubes containing the Cell Lysis Solution with a sterile transfer pipet.

3. Vortex samples for 10 seconds making sure to get rid of all clumps.

4. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate for another half hour, vortex for 5 seconds, and incubate again for half an hour (for a total of one and a half hour of incubation). Samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

Protein Precipitation

1. Cool samples to room temperature.

2. Add 3.33 ml Protein Precipitation Solution to the cell lysate.

3. Vortex vigorously at a high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.

4. Centrifuge at 3800 RPMs and 19ºC for 5 minutes. The precipitated proteins will form a tight white pellet. If the protein pellet is not tight, repeat Step 3 followed by incubation on ice for 5 minutes and then repeat Step 4.

DNA Precipitation

1. Pour the supernatant containing the DNA in one continuous motion (leaving behind the precipitation protein pellet) into a 50 ml tube containing 10 ml of 100% Isopropanol (2- propanol).

2. Mix the samples by inverting gently together on a rotator 50 times to precipitate the DNA. Samples are stable in 100% Isopropanol indefinitely at room temperature.

3. Centrifuge at 3800 RPMs for 3 minutes; the DNA will be visible as a small white pellet.

4. Pour off supernatant in one continuous motion and drain the tube briefly on clean absorbent paper for one minute. Pellet may be loose so pour slowly and watch pellet.

5. Add 5 ml 70% Ethanol and invert tube several times to wash the DNA pellet.

6. Centrifuge at 3800 RPMs for 3 minute. Carefully pour off the ethanol in one continuous motion. Pellet may be loose so pour slowly and watch pellet.

7. Invert and drain the tube on clean absorbent paper and allow sample to air dry 10 minutes.

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[1] Add 5ml RNase (4mg/ml, >80U/ml) directly to 1L Cell Lysis Solution. Date bottle; once RNase has been added, the solution must be used within 8 weeks.

DNA Hydration

1. Add 2 ml DNA Hydration Solution (Adding 1 ml will give a concentration of 350 μg/ml if the total yield of DNA is 350 μg).

2. Rehydrate the DNA by incubating at 65ºC for 1 hour and overnight at room temperature on a rotator. During this time, the DNA will rehydrate and mix uniformly with the DNA Hydration Solution.

3. For storage, sample may be centrifuged at 3800 RPMs and 19ºC for 1 minute and then transferred to a 1.5 ml Nunc tube.

4. Store DNA at 4ºC. For long-term storage, store at -20ºC or -80ºC.

Section 3: CORD BLOOD COLLECTION PROTOCOL

Protocol courtesy of Brigham and Women's Hospital

1. The labor and delivery suites will be equipped with blood collection kits. They will be kept in the East Pod Soiled Utility room since the majority of Harvard Vanguard deliveries occur on this pod.

2. The kit will contain, 1 x 60cc syringe, 1x 18 gauge needle, alcohol wipe, betadine swabs, 2 x10cc red top vacutainer, 1X10cc purple top vacutainer, and 3X10cc green top vacutainers.

3. Each participant in the Viva study will be previously identified before they deliver. A hard copy participant list will be kept in the lounge that will be updated as participants enroll in the study. We will flag these participants through the Harvard Vanguard HAC sheets which will include a pink sheet about the study. The patient is registered and given a BWH card. This card is used to generate all patient labels. If you see the pink sheet generate 5 extra labels. These labels will be put on the vacutainers, and container with the placenta.

4. The baby is delivered. Cord has been clamped. Double glove.

5. Before the placenta is delivered the midwife or MD cleans the cord with betadine. A constant sterile field must be maintained.

6. Put needle and syringe together. Insert the needle into cord and try to collect 55 cc's. The temptation is to draw this volume quickly, but it is essential to cellular work that the volume is drawn slowly so as not to traumatize the cells.

IDEAL COLLECTION: (Tubes should be filled in order listed)

1. 5cc's - red top to BWH blood bank (THIS TUBE IS ONLY FILLED HALF WAY)

2. 10cc's -red top to VIVA container in soiled utility room fridge

3. 10cc's -purple top to VIVA container in soiled utility room fridge

4. 30cc's - 3 green tops need to be stored at room temperature. Place in VIVA box on top of fridge in soiled utility room fridge

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Total 55 cc's DO THE BEST YOU CAN TO FILL ALL

7. The placenta is delivered and is routinely placed in a plastic container. At the discretion of the practitioner the placenta will go to pathology and we will get copy of report. If normal delivery place a sticker on the storage bucket and put in the specimen refrigerator on the Viva shelf. These placenta will not be thrown out till the RA's weigh them and then move them to the discard shelf.

8. RA's from the Channing lab will come to L&D 3 times a day to pick up specimens, replace kits and weigh placentas. After the placenta is weighed it should be placed in the Human discard materials area.