VIVA MID-TEEN SAMPLE PROCESSING

1	Current Viva Mid-Teen collections are intended to include the following: *Mother Mid-Teen Collection* • (1) 10mL EDTA – Purple Top Tube • (1) 10mL Heparin – Green Top Tube • (1) 30mL Conical Tube containing urine *Proband Mid-Teen Collection* • (1) 10mL EDTA – Purple Top Tube • (1) 10mL Heparin – Green Top Tube • (1) 30mL Conical Tube containing urine • (1) PAXgene Blood RNA Tube
2	Upon receipt, follow the Sapphire protocol to properly log in each sample collection per subject. Log in all samples under the "VIVA" study and choose the accurate collection, either "Mother at Mid-Teen" or "Proband Mid-Teen." When receiving samples, be sure the correct subject is selected in Sapphire. Note: VIVA collections can occur at a home or at a clinical site. "Home" is currently not a collection site option in Sapphire. For home collections, the collection site can be left blank.
3	Once all samples have been logged in and properly labeled, proceed with sample processing as described below for each sample type.

10mL EDTA – Purple Top Tube

1	Prepare the tube set-up by properly labeling the following: • (1) 50mL conical tube • (2) NUNC cryotubes for whole blood • (2) NUNC cryotubes for plasma • (1) NUNC cryotube for WBC
2	Mix the blood tube by gently inverting 10-20 times. Transfer 1.8mL of whole blood into each of the labeled cryotubes with a pipette. Note: If the blood tube is less than 50% full when received, DO NOT PREPARE THESE ALIQUOTS and proceed to Step 3.
3	Centrifuge blood tube for 10 minutes at 2,000 RPM and room temperature.
4	While the sample is spinning, add 30mL of RBC Lysis Solution to the labeled 50mL conical tube.
5	Remove sample from the centrifuge and, with a pipette, carefully remove the top plasma layer and transfer it evenly between the labeled cryotubes. Each aliquot should have at least 300µL of plasma. Do not disturb the middle buffy coat.
6	With a pipette, transfer the remaining buffy coat and blood to the prepared 50mL conical tube. Mix by gently inverting the tube 10 times.
7	After mixing, incubate samples for 5 minutes at room temperature (15-25°C). Invert the tube at least once during incubation.
8	Centrifuge sample for 2 minutes at maximum speed.
9	Remove sample from the centrifuge and carefully pour off the supernatant into an appropriate biohazard waste container. Leave approximately 200µL of residual liquid in the tube and vortex vigorously to resuspend the cell pellet in the liquid.
10	Transfer the resuspended WBC to the labeled cryotube. Add 1200µL of NE Buffer and mix by pipetting up and down. Store cryotubes at -80°C until additional analysis is requested.

10mL Heparin – Green Top Tube

1	Prepare the tube set-up by properly labeling the following: • (2) NUNC cryotubes for plasma • (2) NUNC cryotubes for RBC
2	Centrifuge blood tube for 10 minutes at 2,000 RPM and room temperature.
3	Remove sample from the centrifuge and, with a pipette, carefully remove the top plasma layer and transfer it evenly between the labeled cryotubes. Each aliquot should have at least 300µL of plasma.
4	Carefully remove and discard any remaining plasma as well as the buffy coat with a pipette. Be careful not to disturb the bottom red layer containing the red blood cells.
5	Using a disposal transfer pipette, add 3mL of PBS to the blood tube. Mix by gently inverting the tube 10 times. Do not shake or vortex.
6	Centrifuge blood tube for 10 minutes at 2,000 RPM and room temperature.
7	With a pipette, carefully remove and discard the top layer.
8	Repeat steps 5 and 6 to wash the red blood cells again
9	Remove sample from the centrifuge and, with a pipette, carefully remove and discard the entire top layer to ensure no PBS remains. Aliquot the remaining red blood cells evenly between the labeled cryotubes. Each aliquot should contain at least 300µL of RBC.

50mL Conical Tube Containing Urine

1	Prepare the tube set-up by properly labeling the following: • (6) NUNC cryotubes
2	Using a pipette, transfer 1.8mL of urine from the conical tube to each labeled cryotube. Do not transfer more than 1.8mL per cryotube.
3	Discard any remaining urine in the appropriate biohazard waste container.

PAXgene Blood RNA Tube

1	Thoroughly mix each tube by inverting gently 10 times.
2	Store tube at -20°C. If the tube will be stored long-term at -80°C, keep the tube at -20°C for at least 24 hours before transferring to the -80°C freezer.

PROTOCOL REVISION HISTORY

7/26/17	Protocol created
8/29/17	Protocol updated to remove the preparation of FTA cards