CHAPTER 5. SPECIMEN COLLECTION

5.1 Vitamin Levels Assessment

5.1.1 Schedule

Vitamin levels will be assessed while the subject is receiving vitamin supplementation or until the time of transplant:

- 1 month after entry into the study.
- 3- and 6-month follow-up visits.
- 12, 18, and 24 months of age.

If a subject no longer receives the vitamin supplementation because the total bilirubin concentration is <1.5 mg/dL, the vitamin concentrations will be checked at all the above times except at 18 months of age. Blood will be obtained at the study visits before the daily dose of vitamins is given.

The Clinical Research Coordinator (CRC) will call the family prior to the visit to remind them to not give the morning dose of vitamin supplements and that we would like to obtain blood when the infant has not had formula or food for approximately 4 hours.

5.1.2 Testing

The Pediatric CTRC Core Laboratory at The Children's Hospital in Denver will perform the vitamin testing, except for the Proteins Induced by Vitamin K Antagonism or Absence (PIVKA-II). The PIVKA-II sample will be held at the study site and then shipped to the repository at Fisher BioServices on a monthly basis, along with other specimens that are sent to Fisher BioServices.

The priorities for analysis of the vitamin and bile acid levels (if a specimen is insufficient in volume for all to be analyzed), unless otherwise specified by the study site or dictated by clinical care, will be the following order:

1. Vitamins A (retinol) and E (alpha tocopherol)
2. 25-hydroxy-Vitamin D
3. Total serum lipids
4. Retinol binding protein (RBP)
5. PIVKA
6. Total bile acids

5.1.3 Sample Size Requirements

The Pediatric CTRC Core Laboratory at The Children's Hospital in Denver requires serum volumes as follows to conduct specific vitamin tests:

• 500 µl of serum to assay Retinol, Alpha and Gamma Tocopherol, and Total Lipids.
• 300 µl of serum for Retinol Binding Protein (RBP).
• 200 µl of serum for 25OH Vitamin D and Total Bile Acids.

Fisher BioServices requires serum volumes as follows to conduct specific vitamin tests:

• 300 µl of serum for the PIVKA-II assay.

5.1.4 Specimen Labeling

- Prepare three (3) amber vials provided by the Data Coordinating Center (DCC). Label using manifest labels (Form 90). Write subject name, medical record number, and Date Of Birth (DOB) on label. Do **NOT** include study ID number or barcode on these vials. Use a Sharpie or other type of permanent non-smudge marker to write on label. (Securline® makes a marker Marker II/SuperFrost that works well). **Please write legibly.**

  NOTE: To ensure that labels remain attached to vial, label cryovials a few hours before they are to be placed in freezer. Wrapping scotch tape over labels before freezing helps to secure label on vial.

- Prepare one clear "repository" vial, provided by the DCC. Label with the 10th barcoded manifest labels (Form 51, label is pre-identified as "PIVKA"). Some Form 51's may not contain a pre-identified PIVKA label, if this is the case use the 10th label on this manifest anyway. Do **NOT** include any Protected Health Information (PHI) on this vial.

5.1.5 Specimen Collection and Processing

Collection: Draw 3.0cc of blood into a gold-top Serum Separator Tube (SST) vacutainer. Once drawn, cover the vacutainer with aluminum foil to protect it from light.

Centrifugation: Centrifuge the sample to obtain serum.

Aliquot: Aliquot plasma into labeled vials.

- 500 µl into the first amber vial.
- 300 µl into the second amber vial.
- 200 µl into the third amber vial.
- 300 µl into the clear vial.

Store filled cryovials in –70°C freezers.

5.1.6 Shipping Procedures

Amber Vials

Complete the shipping manifest form (Form 90). Use Sharpie or other type of permanent non-smug marker to write on manifest. (Securline® makes a marker Marker II/SuperFrost that works well). **Please write legibly.** You may also attach subject's clinical label to manifest if available.

Ship the three amber vials labeled with PHI only, on dry ice, to Denver using FedEx overnight, along with a copy of Form 90. Do **NOT** send the DCC a copy of Form 90.

NOTE: Shipping Supplies

- Study sites need to supply their own shipping boxes. Denver will return the shipping boxes to the CRC once specimens have been received. If you would like your shipper returned, include a pre-completed Fed-Ex or UPS shipping slip (including your study sites billing information) along with your specimen shipment. Denver will ship the box back to you.
- The DCC does not supply pre-paid Fed-Ex or UPS shipping slips for vitamin levels sent to Denver. Study sites must pay for shipping charges out of their study budgets.

The address of the repository in Denver is:
Pediatric GCRC Core Laboratory
Attn: Peggy Emmett
The Children's Hospital
13123 East 16th Avenue, Room A0922
Aurora, Colorado, 80045

Notify the laboratory by phone (720-777-8209) or fax (720-777-8100) a copy of the completed manifest (Form 90) when a shipment is sent, so the lab can anticipate its arrival. Email may also be sent to: corelab@tchden.org or Emmett.peggy@tchden.org to alert the lab that a specimen has been shipped. **Note:** These emails addresses may not be checked daily.

Overnight shipments should only be sent on Monday, Tuesday and Wednesday; DO NOT SHIP ON THURSDAY OR FRIDAY.

Clear Vial

Complete the shipping manifest form (Form 51). Ship the one clear vial labeled with barcode only, to Fisher BioServices, along with monthly batch shipment of other specimens to Fisher BioServices.

5.1.7 Results

The Denver lab will generate reports that are sent to each study site. Denver's goal is to have the results back to the PI within 7 days or receipt. RBP can take an extra few days. The Denver Lab will make every effort to include the RBP in the 7-day turn-around time. The lab will contact the study site when there is either a delay in getting the results or if any abnormal results are detected. If a problem occurs with the RBP, the other results will be sent without the RBP to the Principal Investigator (PI) as soon as completed. When the RBP is finished, these results will be sent separately.

5.2 Whole Blood for Genetics

Whole blood for genetics is collected as part of PROBE; refer to the PROBE MOO for specifics on collection.

NOTE: If a START subject is transplanted prior to their first birthday, blood for genetics should be collected after the transplant. This specimen can be collected as part of PROBE or START.

5.3 Antibody Titers

Subjects will receive all routine childhood vaccines according to the schedule recommended by the America Academy of Pediatrics (AAP), except that immunizations will not be given for the first 4 weeks after portoenterostomy (a period of time when the corticosteroid/placebo dose is 2-4 mg/kg/day). The normal immunization schedule will then be resumed, with immunizations to be given prior to one year of age being delayed by up to 4 weeks. If there is a need to catch-up with routine immunization schedule, it is anticipated that the catch-up schedule recommended by the Committee on Infectious Diseases of the AAP will be used by the primary care provider.

At the age of 18 months, if the subject has not been transplanted, 3 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. These antibody titers will be done at the study sites local clinical laboratory and paid for out of the study sites study budget.

Results from the antibody titers will be recorded on Form S22V. If serum antibody titers do not achieve protective levels, the information will be provided to the primary care provider so that booster/re-immunization of appropriate antigen(s) is given. Absent titers will also be communicated to the primary care provider, who will treat with re-immunization as directed by the AAP guidelines for catch-up immunization.