CLINIC VISIT
Collection of biologic samples at clinic visit

Prior to any biologic samples being collected, clinic personnel verbally confirms subject identity, name and date of birth, with parents.

Throat Swab:

- **Discontinued among antibody negative participants December 2015**
- **Discontinued among antibody positive participants July 2017**
- Label viral media vial with ID#, date, and sample # using cryopens on cryo lab tape.
- For young children, have child sit on parents lap facing forward.
- Ask participant to open mouth, depress tongue for better visualization if necessary, expose tonsil area.
- Swab each tonsil with sterile cotton tipped applicator.
- Place cotton tipped applicator into M4 viral media, stir gently assuring cotton tip applicator contacts media beads.
- Gently press and rotate cotton tip on side of vial to express all media.
- Dispose of applicator in waste receptacle.
- Transfer vial to lab for documentation and storage at -70C.

Saliva:

- **Discontinued for all participants December, 2015**
- Participant/parent chooses sugar-free lollipop
- Label collection cup, ID# +S
- Participant sucks on lollipop in mouth to stimulate salivation.
- Participant spits saliva into sterile collection cup. Obtain 3cc.
- If unable to spit (very young children):
    - Utilize sterile ear/ulcer syringe (bulb syringe)
    - Have child/parent hold lollipop in mouth, depress sides of syringe
- Insert tip of syringe into side of mouth or under tongue, release syringe allowing aspiration of saliva. Transfer to sterile collection cup. Obtain 3 cc.
- Place lid on cup, transfer to lab for aliquotting, documentation and storage at -70C.

Rectal swab:

- **Obtained ONLY when parent is present**
- **Discontinued for all participants December, 2015**
- Label viral medial vial with ID#, date and sample # using cryopens on Cryo lab tape.
  Infants:
    - Have parent lie child in supine position on exam table.
    - Have parents remove diaper and lift child's legs exposing rectum.

- Use sterile cotton tipped applicator, gently swab rectal area try to obtain small amt. of fecal material. If no fecal material present, gently insert tip of cotton tipped applicator into rectal opening no more than the length of the cotton on the applicator.
- Remove applicator gently. Immediately place applicator into M4 viral media, stir gently assuring cotton tip applicator contacts media beads.
- Gently press and rotate cotton tip on side of vial to express all media.
- Dispose applicator in waste receptacle.
- Transfer vial to lab for documentation and storage at -70C freezer.

Children:

- Have child lie prone over parents lap.
- Have parents gently expose buttocks.
- Verbally notify child of every step taken prior to touching them.
- Spread buttocks apart exposing rectum.
- Gently wipe rectal area with sterile cotton applicator, try to obtain fecal material. If no fecal material present, gently insert tip of cotton tipped applicator into the rectum no farther than the length of the cotton covering.
- Remove gently
- Immediately place applicator into M4 viral media, stir gently assuring cotton tip applicator contacts media beads.
- Gently press and rotate cotton tip on side of vial to express all media.
- Dispose applicator in waste receptacle.
- Transfer vial to lab for documentation and storage at -70C freezer.

Participant sample collection:

- When participant is approximately 10 years old and older they can obtain their own rectal culture preferably with parent assistance.
- Child is to obtain sample in the participant bathroom.
- **Instruct participant:**

1. After collecting urine sample (if needed) take one sterile cotton tip applicator, do not touch applicator to anything else.
2. Reach behind and wipe rectal area, anus, with cotton tip applicator then immediately place applicator into viral media.
3. Use language the child can understand. “Wipe your bottom right where you poop” and tell them “It’s OK, actually better if they get some poop on the q-tip”.

- Immediately place applicator into M4 viral media, stir gently assuring cotton tip applicator contacts media beads.
- Gently press and rotate cotton tip on side of vial to express all media.
- Dispose applicator in waste receptacle.
- Transfer vial to lab for documentation and storage at -70C freezer.

Height and Weight:

For infants and toddler less than 2 years of age, do not measure height

Height:

- Participant stands with back against Genentech Stadiometer, ACCUSTAT. Ask participant to stand with back against the ACCUSTAT. Knees should be straight, with heels on floor and with head, shoulder blades, buttocks, and heels touching the wall.
- Grasp headboard firmly near knob and loosen knot. Ask the participant to look straight ahead.
- Lower headboard to rest against crown of the head and tighten knob.
- Read measurement to the nearest 0.1 cm as noted by the thin black line of the headboard on the measuring backboard.
- Document height in centimeters and inches on the clinic visit sheet.
- Inform participant (or parent) of the recorded height in feet and inches.

Weight:

Infants:

- Infant should be weighed in comfortably minimally clothed manner (onsie or t-shirt and diaper). Shoes are to be removed.
- Infant scale is to be covered with only a clean paper shield.
- Place infant in the center of the scale. Parents can place child in the position most comfortable for the child, sitting upright or lying supine. All body parts should be in the scale tray field.
- Document weight in kilograms and pounds on clinic visit sheet.
- Inform parent of recorded weight in pounds.

Older Children and Adults.

- Make sure Healthometer Physician scale is on a hard, level surface. Press "START" Beep will sound and display will say "WAIT".
- Wait until 2 beeps are heard and display reads 0.0.
- Have participant remove shoes and step onto weighing mat making sure feet are directly in the middle of the mat.
- Have participant stand quietly until another beep is heard and a steady weight is displayed. The weight can be displayed in either Kg or Lbs.
- Document the weight in kilograms on the clinic visit sheet. Pressing the Kg/Lb button will display the weight in the alternate format of what is displayed.
- Inform the participant of the recorded weight in pounds.

Abdominal (Waist) Circumference:

- **This measurement implemented June, 2010**
- Waist circumference measurement will be taken for participants aged 2 years and older.

- Instruct the participant to gather his or her shirt above the waist, cross the arms, and place the hands on opposite shoulders-demonstrate the desired position of the arms. It may help to say think of giving yourself a hug.
- If necessary, ask the participant to lower the pants and underclothing to slightly below the waist. **Always tell the participant what you are going to do before you do it**.
- Stand or sit at the participant's side.
- Palpate the hip area to locate the right ilium of the pelvis-Take the measurement at this level. Extend the measuring tape around the waist, positioning the tape in a horizontal plane at the level of the iliac crest.
- Check that the tape sits parallel to the floor and lies snug but does not compress the skin. Always position the zero end of the tape below the section containing the measurement value.
- Take the measurement to the nearest 0.1 cm at the end of normal expiration.
- Record the result in centimeters.
- Repeat the process for a second measurement.

BIA Measurement using TANITA Body Composition Device

- **This measurement implemented April, 2018**
- DAISY will use the Tanita DC-430U model
- Measurement taken at every DAISY clinic visit
- Ask participant to remove shoes and socks
- Have participant step onto the electrode platform, so that bare feet have maximal contact with all four electrode areas, with arms hanging straight down.
- Staff should stand clear of the device and participant to ensure accuracy
- Device will display kilograms of body fat and body fat%
- Document measurements on the clinic visit sheet for the participant

Collect Urine

Discontinued among antibody negative participants July, 2017

Infants and Toddlers not potty trained:

- Cotton balls are mailed to the family the week prior to the visit.
- Family is instructed to make sure child has extra fluids to promote urination.
- Family is instructed to put cotton ball in the diaper area most likely to become wet during urination (front for boys, back for girls).
- At the time of the rectal swab collection, when the diaper is opened by the parent the wet cotton balls are placed in small 3X4 ziplock bag labeled with ID# and U.
- Urine soaked cotton balls are transferred to the lab for processing, storage and database documentation.

Participant sample collection:

- Parent or participant are instructed prior to the visit to make sure the participant has extra fluids to promote urination.
- When participant needs to urinate a sterile specimen cup will be given to parent or participant. Specimen cup is to be labeled with ID# and U.

Males: Instruct to void directly into the urine cup approximately 1" of urine, secure cap on the cup and leave in the bathroom pass-through door. Lab staff will process immediately.
Females: A clean dry urine collection "hat" will be placed by the clinic staff on the toilet seat. Instruct to void into the urine collection container and leave the container in place. Clinic staff transfers urine from the collection container to the sterile labeled specimen cup to approximately 1", secure cap and place in bathroom pass-through door. Lab staff will process immediately.

Blood Draw

- Collect blood sample at every DAISY clinic visit to evaluate primary outcome: islet autoantibody detection.
- Parent and/or participant may decide to have participant sit on a parent lab, lie down on exam table or sit alone for the blood draw procedure.
- Assistance may be needed to keep arm still and stable during the blood draw procedure- a second staff member should be utilized. One hand under the elbow to prevent flexion, one hand at the wrist to keep the arm still.
- All tubes are to be labeled with participant ID # and date at the time of sample collection.
- Blood collection tubes are held in a tube rack once filled.
- A different rack should be used for each individual, when possible, use a different color of ink to label tubes as well.
- For enrolled DAISY participants, pre-printed ID labels are in the chart. Each tube should have a label applied immediately.
- Blood collection priority: SST, EDTA, Heparin, ABI, (see BloodAllocation table) located in each clinic room).
- Blood volume limits: 3cc/kg weight of participant. Standard blood volume is 30cc.
- Maximum of 2 attempts to collect blood.

EMLA numbing cream:

- EMLA may be applied by clinic staff if requested.
- Must be applied 30 minutes prior to blood draw to be effective, using Tegaderm to contain cream in the antecubital area.

Ethyl Chloride Spray:

- Ethyl chloride spray may be applied just prior to the blood draw.
- Indications: Ethyl Chloride is a vapo-coolant (skin refrigerant) intended to topical application to control pain associated with injections and IV placement.
- Contraindications: Ethyl Chloride is contraindicated in individuals with a history of hypersensitivity or previous allergic reaction.

Blood draw procedure:

- Explain process to participant and/or parent if subject is too young.
- Explain the sensation of the spray is very cold, if using Ethyl Chloride.
- Remove Tegaderm and clean EMLA cream from antecubital area, if used.
- Assess the site for the optimal insertion site.
- Assure all supplies are prepared.
- Apply tourniquet.
- Clean needle insertion site with alcohol pad.

- If using spray, aim spray at intended needle insertion area. Spray the area with Ethyl Chloride for 4-10 seconds from a distance of 3-9 inches. Stop spraying when skin begins turning white, no more than 10 seconds elapsed time.
- Quickly introduce the needle with skin pulled taut.
- Obtain appropriate amount of blood for this visit.
- Remove tourniquet.
- Remove needle, cover insertion site with cotton ball or gauze applying mild pressure.
- Apply Band-Aid or Coban wrap.
- Aliquot blood into appropriate blood collection tubes per protocol, inverting tubes several times to mix additive with blood sample.

Random Glucose (Antibody positive participants only)

- A random glucose measurement is completed for all islet antibody positive participants and anyone who has been persistently positive in the past.
- A small amount of blood from the blood draw procedure is transferred into an EDTA microtainer.
- The microtainer is transferred to the lab for immediate measurement, using the ContourNext One glucometer.
- The clinic staff will return to the lab for the random glucose measurement result.
- The lab staff records the result into the Glucometer Sample Logbook and the clinic visit sheet for the participant.
- If the meter displays HI, the measurement will be completed again.
- If the results is >100mg/dL for a fasting participant or >140 mg/dL for a non-fasting participant, the blood glucose will be measured using an alternative device:
    - YSI 2000 STAT: April, 2007-December, 2017
    - HemoCue 201 Glucose: July, 2018-current

HemoglobinAIC (Antibody positive participants only)

- Hemoglobin A1c (HbA1c) is a lab test that measures the average blood sugar over the last few months.
- This test is done on all islet antibody positive participants at each visit and anyone who has been persistently positive in the past.
- A small amount of blood from the blood draw procedure is transferred into an EDTA microtainer.
- The microtainer is transferred to the lab for measurement using the DCA Vantage device.
- The clinic staff will return to the lab for the HbA1c measurement result.
- If the result is >6.3 the clinic staff will contact the study investigator or BDC pediatric physician on-call, to discuss the results with the participant and/or family.