

DAISY LAB

A. What samples to collect

The samples to be collected depend on several factors: what affiliated studies the individual is enrolled in (IVY, CEDAR), what cohort the individual belongs to (SOC, NEC, NOC, CNEC, FAM), and what type of visit it is (initial, annual, positive).

DAISY

(If individual is also in IVY or CEDAR then also see the section for that study)

IVY: Includes moderate and high risk NECs, NOCs and SOCs enrolled prior to the age of 2 years, and all children who test positive for diabetes autoantibodies.

CEDAR: All of the study subjects and siblings under the age of 10 that we see in clinic are tested for the celiac autoantibody (TG) which is the primary outcome measure for the CEDAR study, but only those with a cohort of CEDAR or CNEC are *exclusively* in the CEDAR study. Fewer samples are collected on subjects who are *exclusively* enrolled in CEDAR (see section on CEDAR).

Parents and older siblings are only tested for TG if they report having had any of the celiac symptoms in the year prior to the clinic visit or if they are diabetic. TG will also be tested on otherwise ineligible family members if the family specifically requests that it be done.

Initial visit. An initial DAISY visit that is not IVY, not CEDAR and not DPT, will generally be for a new SOC who is between 2 and 3 years old. All other new subjects tend to be part of the IVY protocol.

The following specimens should be collected:

Saliva: 1-5cc of saliva collected into specimen cup

Viral Cultures: Collected from throat (1) and rectum (2) using cotton swabs. Swabs are then 'swirled' into tubes containing viral media.

Whole blood: The purpose of the whole blood is twofold. It is important to have the HLA type of all subjects and family members, and it is important to have at least one sample of whole blood stored as well. If we do not have the study subject's HLA type, then 0.5ml of whole blood is collected to send to Roche laboratories, and 1.25ml is collected for storage. The Roche whole blood is labeled with the DAISY ID and subject initials, and the tube for whole blood storage is labeled in the same way as other tubes for storage (see below). Generally we need to collect both samples for SOC initial visit, but most NECs and NOCs should already have an HLA type and therefore a sample does not need to be sent to Roche, **but we still need to collect 1.25ml for storage**. Sometimes, especially with very young kids, it is not possible to get the whole blood. If this is the case, it should be noted in the chart and collected at the next visit. If for any reason you are in doubt, the HLA type or risk is printed on the clinic visit sheet. If this field is blank, we need HLA! ***This is one of the most***

commonly made mistakes—please double check! At initial visits, other family members usually also have their blood drawn. This is covered in more detail below.

Serum (Red top): Blood collected in red tiger-top vacutainer. Approximately 3ccs is the absolute minimum that can be collected, but in most cases, 7-10ccs are collected. Vacutainer should be left at room temperature until the blood clots. Serum is used to test the blood for diabetes and celiac autoantibodies, and is therefore the highest priority tube.

Whole blood (Extra purple top): This blood is to go to the BDC for DNA extraction. The BDC is conducting additional genetic testing as a part of their on-going research. This is collected in a purple top vacutainer (often referred to as the ‘extra purple top’). The blood is not processed in any way, but is recorded as collected and bar-coded for identification. It is stored in the fridge until taken to the BDC. This is a one-time collection – once we have collected it on a person, it does not need to be collected again. The clinic sheet should indicate whether or not a sample has been collected in the past. This may not be collected at an initial visit in small children due to the volumes of blood needed.

ABI tube (mRNA): Only collect this tube if you can aliquot 2.5cc of blood into this tube. Samples are frozen and stored at -70 until shipped to another lab for mRNA extraction and expression assays. This may not be collected at an initial visit in small children due to volumes of blood needed.

Annual Clinic Visit. An annual clinic visit that is not IVY and not CEDAR will generally be for older moderate or low risk NECs and NOCs. Most older SOC's are not in IVY or CEDAR, please refer to the clinic visit sheet.

Saliva

Viral cultures

Serum

Plasma (Regular purple top): Generally 5-10ccs are collected. This blood is used to collect plasma and buffy coat for storage.

Whole blood (regular purple top): The purple top is used to collect whole blood for Roche HLA testing and storage and, after centrifuging, plasma and buffy coat for storage.

Whole blood (Extra purple top): This blood is to go to the BDC for DNA extraction. The BDC is conducting additional genetic testing as a part of their on-going research. This is collected in a purple top vacutainer (often referred to as the ‘extra purple top’). The blood is not processed in any way, but is recorded as collected and bar-coded for identification. It is stored in the fridge until taken to the BDC. This is a one-time collection – once we have collected it on a person, it does not need to be collected again. The clinic sheet should indicate whether or not a sample has been collected in the past.

ABI tube (mRNA): 2.5cc of blood should be collected into this tube at every visit.

Family Members. We try to draw blood at least once from all family members of study subjects. Usually this is done at the initial clinic visit, but if certain family members are not present at the initial visit, we try to get them at a future visit. The following samples are collected from family members:

Serum: Serum is collected in the red tiger-top tube and is used to test for diabetes autoantibodies.

Whole blood (regular purple top): The purple top is used to collect whole blood for Roche HLA testing and storage and, after centrifuging, plasma and buffy coat for storage.

Whole blood (extra purple top): This blood is to go to the BDC for DNA extraction. The BDC is conducting additional genetic testing as a part of their on-going research. This is collected in a purple top vacutainer (often referred to as the ‘extra purple top’). The blood is not processed in any way, but is recorded as collected and bar-coded for identification. It is stored in the fridge until taken to the BDC. This is a one-time collection – once we have collected it on a person, it does not need to be collected again. The clinic sheet should indicate whether or not a sample has been collected in the past.

Positive visits. Study subjects who test positive for diabetes autoantibodies become part of the IVY study. All visits for kids who have ever tested positive are IVY positive visits (see that section below). This includes siblings of study subjects who test positive for diabetes autoantibodies. Parents who test positive for diabetes autoantibodies are not enrolled in IVY. Testing positive for celiac autoantibodies (TG) does not make anyone eligible for IVY.

IVY

The IVY study generally includes moderate and high risk NECs, all NOCs and SOCs who were less than 2 years old at the time of enrollment, and all children who test positive for antibodies regardless of cohort or age (including family members). The IVY status of an individual is indicated at the top of the clinic visit sheet.

Initial IVY visit. An initial IVY visit will occur at 9 months of age for NECs/NOCs, or at up to 2 years of age for SOCs.

Saliva: 1-5cc of saliva collected into specimen cup

Urine: Collected directly into specimen cup or indirectly using cotton balls placed inside the diaper. We can’t store more than ~5ccs so even if it’s just a little, we can use it! If it’s impossible to get in clinic (cotton balls are too dry or kid just can’t/won’t go), we may still be able to get it. If the family lives in the Denver area, we can send a ‘urine kit’ home with them, which they can bring back themselves or it can be picked up at their home by Jennifer.

Viral Cultures: Collected from throat (1) and rectum (2) using cotton swabs. Swabs are then ‘swirled’ into tubes containing viral media.

Whole blood: The purpose of the whole blood is twofold. It is important to have the HLA type of all subjects and family members, and it is important to have at least one sample of whole blood stored as well. If we do not have the study subject’s HLA type, then 0.5ml of whole blood is collected to send to Roche laboratories, and 1.25ml is collected for storage. The Roche whole blood is labeled with the DAISY ID and subject initials, and the tube for whole blood storage is labeled in the same way as other tubes for storage (see below).

Generally we need to collect both samples for SOC initial visit, but most NECs and NOCs should already have an HLA type and therefore a sample does not need to be sent to Roche, **but we still need to collect 1.25ml for storage.** Sometimes, especially with very young kids, it is not possible to get the whole blood. If this is the case, it should be noted in the chart and collected at the next visit. At initial visits, other family members usually also have their blood drawn. This is covered in more detail below.

Serum (Red top): Blood collected in red tiger-to vacutainer. Approximately 3ccs is the absolute minimum that can be collected, but in most cases, 7-10ccs are collected. Vacutainer

should be left at room temperature until the blood clots. Serum is used to test the blood for diabetes and celiac autoantibodies, and is therefore the highest priority tube.

Plasma (Foil-wrapped Purple top): Blood collected in foil-wrapped purple top vacutainer. In order to get ALL the IVY blood, a minimum of 5ccs is needed. However, even 2ccs is better than nothing. In most cases, 9-10ccs are collected. This tube is used to collect plasma for testing at the CRC and for Dr. Awad, as well as buffy coat.

Plasma (Foil-wrapped Green top): Blood collected in foil-wrapped green top vacutainer. 1cc is sufficient for testing, but in most cases 3ccs is ideal. This tube is used to collect red blood cells (RBCs) and plasma for testing by Dr. Deutsch.

ABI tube (mRNA): Only collect this tube if you can aliquot 2.5cc of blood into this tube. Samples are frozen and stored at -70 until shipped to another lab for mRNA extraction and expression assays. This may not be collected at an initial visit in small children due to volumes of blood needed.

ALL IVY PLASMA TUBES MUST BE KEPT COLD, AWAY FROM LIGHT, AND PROCESSED WITHIN A HALF-HOUR! Keep blood in the fridge until ready for centrifuging. Blood must be processed within one hour of being drawn.

Annual IVY Visits. The same samples are to be collected at these visits with the exception of the whole blood sample (for Roche and storage) *unless it was missed at the initial visit* (if it was missed it should be so indicated in the clinic chart). The whole blood sample for the BDC is generally collected at an annual visit, and not at an initial IVY visit due to the small size of the child and the large volumes of blood needed.

Saliva

Urine

Viral Cultures:

Serum (red top)

Plasma (foil-wrapped purple top)

Plasma (foil-wrapped green top)

Whole blood (Extra purple top): This blood is to go to the BDC for DNA extraction. The BDC is conducting additional genetic testing as a part of their on-going research. This is collected in a purple top vacutainer (often referred to as the ‘extra purple top’). The blood is not processed in any way, but is recorded as collected and bar-coded for identification. It is stored in the fridge until taken to the BDC. This is a one-time collection – once we have collected it on a person, it does not need to be collected again. The clinic sheet should indicate whether or not a sample has been collected in the past.

ABI tube (mRNA): 2.5cc of blood should be collected into this tube at every visit.

Positive IVY visits. After a study subject or sibling is confirmed positive for diabetes auto-antibodies, they are enrolled in the IVY study. Positive kids are usually seen every 3-6 months instead of annually. These visits are treated like a regular IVY visit, with the addition of the following:

Random glucose

A1C

These tests do not require that any additional blood be drawn, but the phlebotomist should be prepared to perform these tests. Since quality control samples are automatically sent for retesting of antibodies on any positive subject, *4 QC samples are collected* instead of the usual 3 (more on QCs below).

CEDAR

All of the study subjects and siblings under the age of 10 that we see in clinic are tested for the celiac autoantibody (TG) which is the primary outcome measure for the CEDAR study, but only those with a cohort of CEDAR or CNEC are *exclusively* in the CEDAR study. Fewer samples are collected on subjects who are *exclusively* enrolled in CEDAR.

Parents and older siblings are only tested for TG if they report having had any of the celiac symptoms in the year prior to the clinic visit or if they are diabetic. TG will also be tested on otherwise ineligible family members if the family specifically requests that it be done.

Initial and Annual Visits: Currently enrolled CEDAR subjects are listed as being in the cohort 'CNEC'. These are children who were born at St. Joseph's hospital and who agreed to the genetic screening. These children are not at increased risk for diabetes, but because of their HLA type, they are at increased risk for celiac disease. At one time these children were seen only once every two years, but currently they are being seen yearly, the same as DAISY subjects. Fewer specimens are collected for CEDAR subjects:

Serum: For testing of diabetes and celiac autoantibodies

Plasma (regular purple top): For storage of plasma and buffy coat

Note that only blood samples are collected. Saliva, viral cultures, and urine are NOT collected from CEDAR subjects.

Positive Visits. After testing positive for TG the option is given to come in for a redraw visit to see if the result is persistent, or to be referred for a small bowel biopsy at Children's Hospital. At visits subsequent to the initial positive TG visit, *4 QC samples are collected* instead of the usual 3. Refer to the clinic visit sheet.