BLOOD COLLECTION AND PROCESSING

TABLE OF CONTENTS

I. INTRODUCTION

The phlebotomy station is designed to obtain blood samples to: (1) establish cell lines as a source of DNA for genotyping; (2) test for autoantibodies in affected siblings; and (3) establish a plasma and serum storage repository for future assays. It is potentially a difficult station because of the anxiety caused by blood collection and the potential for injury. However, if carefully and professionally done, it provides a positive, safe experience for the participant. Blood collection will take approximately 10 to 15 minutes of the participant's time. This protocol is designed as a guide for the nurses and technicians at the clinics.

II. LABORATORY QUALITY CONTROL

A split sample (*i.e.*, duplicate sample) program is used to determine how well the network laboratories can reproduce autoantibody measurements and DNA yields from the EDTA cell pack. In addition, a repository of quality control (QC) samples is created for future assays of interest to the T1DGC investigators.

The purpose of the split sample program is to produce data to: (1) identify problems as they occur, and to communicate this information to the clinic and laboratory for corrective action; and (2) document the performance of the entire process (*i.e.*, sample collection, preparation, processing, shipping, and analytical performance).

The quality control procedures consist of providing the laboratories with duplicate samples, one sample labeled with the participant ID and the other with a fictitious (quality control or QC) ID. Each of the clinics collects a QC tube on an approximate 5% sample of the participants for serum samples and 5% for plasma samples. All QC participants must be at least 16 years old (or large for age) due to the additional volume of blood to be collected. (See **Chapter VIII**, *Quality Control*, for specific guidelines and rationale.)

The Clinic Coordinator identifies the QC participants and provides this information to the nurse or technician at the beginning of each clinic week. A *T1DGC Participant and QC Selection Log* has been developed to assist in the selection and tracking of QC participants.

In the event that the additional sample cannot be collected on the identified participant, the nurse or technician collects the QC sample on the **next appropriate participant**. The participant need not be told that he/she is having extra blood collected for QC. The consent form covers the maximum amount of blood to be collected.

III. DESCRIPTION

A. Assays

The following autoantibody assays are performed on the serum samples from the proband, the affected sibling(s), and cases: IA-2_{ic}, GAD65, TPO, TG, H^+/K^+ ATPase, and ZnT8. The measurement methods for each assay are provided in the data documentation for the phenotypic data set and the rationale for assessment is provided in the *T1DGC Protocol*.

B. Recommendations

The following procedures are designed to standardize sample collection.

1. Blood is collected with the participant in a seated position. The reclining position can be used if desired or if participant has a history of fainting during blood collection.

2. Participants are instructed to drink plenty of water during the 24 hours prior to the clinic exam. Blood collection is easier if participants are well hydrated.

3. No restrictions are required for fasting, vigorous activity, or smoking the day of the exam.

4. Blood collection occurs after the questionnaires for eligible participants have been completed.

5. Participants are encouraged to engage in brisk exercise (*e.g.*, walk up and down a flight of stairs or permit children to run outside) prior to blood collection to increase the number of circulating lymphocytes.

C. General Preparation

The blood collection should take place in an isolated room or one enclosed by dividers. Temperature should be 65-75^{o}F (18-24^{o}C). There should be no direct sunlight on samples.

The room should be equipped with all the necessary supplies. (Appendix A provides a list of all blood collection, aliquoting and sample shipment supplies.) A table or counter should be prepared with the materials and vials needed for blood processing and aliquoting. The centrifuge, refrigerator and freezer should be nearby.

Clinic staff records the temperature of the freezer(s) containing T1DGC samples on the *T1DGC Daily Freezer Temperature Log* each day the clinic is in operation. Any values that are outside the specified range are circled. The expected temperatures are -75^{o} to -65^{o}C, but -20 ^{o}C is acceptable for short-term storage (*i.e.*, one to two months). The *T1DGC Daily Freezer Temperature Log* is sent to the Regional Network Center at the end of each month or as requested.

This station is staffed with a nurse or technician with documented class time and experience in phlebotomy. The technician should be properly attired; gloves must be used at all times while processing blood samples. Certification in T1DGC procedures occurs during or following a training session and prior to data collection. Recertification will occur annually or as requested by the Regional Network Center.

D. Blood Volume

All participants under the age of 16 years have 19.9 ml (20.9 ml in the United Kingdom) of blood collected; this is less than 1.5 tablespoons. All participants aged 16 or older will have 27.4 ml (29.4 ml in the United Kingdom) of blood collected; this is less than 2

tablespoons. The tubes to be used are listed below, in priority order:

- one 7.5-ml green top (sodium heparin) tube OR one 8.5-ml yellow top (CPDA) tube;
- one 7.5-ml red top (serum) tube;
- one 4.9-ml purple top (EDTA plasma) tube; and
- one additional 7.5-ml green top (sodium heparin) tube OR one 8.5-ml yellow top (CPDA) tube **in participants 16 years or older**.

Due to the volume of blood to be collected, QC participants should be at least 16 years old (or large for age). Blood collection is split across two individuals to minimize the burden for any one participant. The QC participants are referred to as QC-Red and QC-Purple. A QC-Red participant **must** be a proband, an affected sibling, or a case; a QC-Purple participant can be any participant in the affected sibling pair (ASP) or trio family or a control participant. The QC participants selected have only one additional volume of blood collected, as indicated below, using the following tubes:

QC-Red: one additional 7.5-ml red top (serum) tube for autoantibodies and serum storage; **OR**

QC-Purple: one additional 4.9-ml purple top (EDTA) tube for plasma storage and DNA extraction from the cell pack.

Thus, a QC-Red participant has 5 tubes collected, totaling 34.9 ml (36.9 ml in United Kingdom) or approximately 2.5 tablespoons of blood. A QC-Purple participant has 5 tubes of blood collected, totaling 32.3 ml (34.3 ml in United Kingdom).

IV. BLOOD COLLECTION PROCEDURES

The following steps outline the basic procedures for blood collection in the T1DGC study.

1. Confirm that you have the correct ID labels for each participant. Locate the *T1DGC Blood Collection Form: Original Collection* for each participant in the family and check that it has a bar-coded Participant ID label on each page of the form. There is a separate *T1DGC Blood Collection Form: Original Collection* for the case-control collection study. (Appendix B provides instructions for completion of the blood collection form.) Confirm that the aliquot ID labels for samples match the participant ID on the exam form. Record the nurse/technician ID on page 5 of the form.

2. Before collecting blood, ask the participant whether he/she has any bleeding disorders. If the participant reports a history of bleeding disorders, the participant should be sampled under the supervision of a physician.

3. Explain the procedure. An example might be: "I am going to take some blood from the vein in your arm. The purpose of this is to check levels of autoantibodies (affected participants only) and provide samples for DNA analysis. I will be taking three tubes -- about one and a half tablespoons of blood. Are there any questions?" (**NOTE:** The number of tubes and the total amount of blood collected varies depending on whether the participant is older than 16 years and is selected for QC. This script should be adjusted accordingly.)

4. If the participant asks if he/she will receive results, direct the participant to ask the Clinic Coordinator. This will vary between networks.

5. Identify the best available vein. Palpate and trace the path of veins several times with the index finger. If veins are not readily apparent, have participant close his/her fist or lower the extremity over the arm of the chair to allow the veins to fill to capacity.

6. Use a tourniquet to increase venous filling, leaving it on for the shortest time possible.

7. Cleanse the venipuncture site. Allow the area to dry to prevent possible hemolysis of the sample and a burning sensation to the patient when the venipuncture is performed.

8. Use of Sarstedt tubes:
   a. The tube can be used as a vacutainer (by pulling the plunger at the base of the tube down and snapping it off) or as a syringe (by using the plunger to draw blood into the tube).
   b. Insert the **first blood collection tube (7.5-ml green top or 8.5-ml yellow top)** into the holder.
   c. If a tube has been previously or unsuccessfully used, it should be discarded in a proper container; it should not be re-used.
   d. Inspect the tip of the needle visually to determine that it is free of hooks at the end of the point, and that its opening is clear of any small particles that would obstruct the flow of blood. The needle must be sterile. Do not use a needle from a package that is broken or contaminated in any way.

9. Perform the venipuncture, entering the vein in a smooth continuous motion.
   a. Remove the tourniquet as soon as possible. Once the collection has started, do not change the position of the tube until it is withdrawn from the needle. During the procedure, try not to allow the contents of the tube to contact the cap.
   b. Fill the tube as completely as possible. Partially filled tubes should be avoided. **However, if this occurs, do not discard the partially filled tubes.**
   c. When the blood flow ceases or the tube is filled, remove the tube from the holder.
   d. Immediately and thoroughly mix the contents of **ALL** tubes by gently inverting eight (8) times.
   e. To prevent hemolysis, avoid jarring or shaking the tube. Put the tube into a rack or jacket pocket; do not lay on table. NOTE: Hemolysis is the

alteration, dissolution or destruction of red blood cells in such a manner that hemoglobin is liberated into the medium in which the cells are suspended. Hemolysis can distort the results of some assays and can compromise the quality and use of storage samples.

f. To obtain additional samples from all participants, insert the **next tubes (the 7.5-ml red top tube followed by the 4.9-ml purple top tube)** into holder and repeat procedure.

g. For adults (those at least 16 years old) only, insert **a second 7.5-ml green top tube or 8.5-ml yellow top tube into holder and repeat procedure**.

h. For QC-Red participants, insert **a second 7.5-ml red top tube into holder and repeat procedure**. For QC-Purple participants, insert **a second 4.9-ml purple top tube into holder and repeat procedure**.

i. Record the time that blood was collected on the *T1DGC Blood Collection Form*.

10. Remove the needle quickly and immediately after filling all tubes. Have participant hold sterile pad firmly for one to two minutes to prevent a hematoma. Discard needle into biohazard box (*i.e.*, sharps container).

11. If no staff member is able to obtain a blood sample, the *Blood Collection Form* should be completed as follows:
    a. record the date blood collection was attempted for exam date on page 1 and the nurse/technician ID on page 5; and
    b. mark “no” for question 2 (“Was any blood collected?”) and record “Staff unable to obtain sample” for the reason.

12. If the participant refuses to have any blood collected, the *Blood Collection Form* should be completed as follows:
    a. record the date blood collection was attempted on page 1 and the nurse/technician ID on page 5; and

b. mark "no" for question 2 ("Was any blood collected?") and record "Refused" for the reason.

Clinics will not be reimbursed for participants who refuse to have blood collected since the study aims cannot be achieved without a blood sample from which DNA can be extracted. If the proband and/or affected sibling in an ASP family refuses to have blood collected, the family is ineligible and blood samples should not be obtained from other family members. For this reason, blood collection for the proband and/or affected sibling should precede collection for parents and unaffected siblings whenever possible. In trio families, the family is ineligible if any family member refuses to have blood collected. The case-control study is based on individual participants, so if a case or control refuses, he/she is ineligible.

V. BLOOD PROCESSING

A. Description

The proper processing of the collected samples is critical because deviation from the protocol can significantly affect the future use of the samples. It is particularly important that time deadlines in handling are observed and that samples are not left open to the atmosphere longer than necessary.

A total of ninety (90) minutes is permitted between blood collection and final placement of serum and plasma samples in the freezer. Note that this does not apply to the green top or yellow top tubes or to the EDTA cell pack, which are maintained at room temperature and shipped daily to the DNA Repository.

B. Procedure

1. Immediately after collecting the sample, affix one of the large labels with the participant's ID to each of the tubes to identify the samples belonging to each participant. The large labels and smaller aliquot labels are color-coded for each family member as follows: father - blue; mother - pink; proband – purple; affected sibling(s) – green; unaffected sibling(s) – yellow; case – orange; and control – gray.

2. Place the green top or yellow top tube(s) in a test tube rack. Maintain at room temperature until there is time to prepare the green-top tubes for daily shipping. **DO NOT PROCESS IN CENTRIFUGE AND DO NOT REFRIGERATE AT ANY TIME.** If participant has not consented to creation of a cell line, affix the pre-printed label that indicates "DNA Only – No Cell Line" to the participant's green top or yellow top tube(s).

3. Place the red top tube in a test tube rack. Allow the sample to clot by standing for at least 30 minutes but not more than 60 minutes at room temperature (65-75^{o}F; 18-24^{o} C).

4. Immediately place the purple top tube into a container of water and ice. Cool the samples on water and ice for 30 minutes but not more than 60 minutes.

5. Do not let **any** of the samples stand in direct sunlight or at extreme temperatures.

6. Centrifuge the red top and purple top tubes, following manufacturer instructions.

7. Record the nurse/technician ID for the person processing the samples on page 5 of the *Blood Collection Form*

VI. ALIQUOTING

A. Preparation

The red top tube and the purple top tube collected for each participant will be aliquoted into a total of 9 cryovials. Refer to "Labeling Aliquot Vials" (Section VI.B), "Aliquoting Samples" (Section VI.C) and "Blood Collection Flow Charts" (Appendix C) for assistance in this process.

The green top (sodium heparin) or yellow top (CPDA) tubes are **not** processed at the clinics; the green top or yellow top tubes are shipped **daily** to the DNA Repository for processing. Store the green top or yellow top tubes in a rack at room temperature until

shipped. **DO NOT REFRIGERATE, CENTRIFUGE OR ALIQUOT.**

The nurse or technician prepares the work area by laying out the plastic transfer pipettes and aliquoting vials and tubes. Affix the small ID labels to each sample vial as indicated in the following table and diagram.

Serum and plasma samples are identified by using color-coded polypropylene caps for the cryovials. Red caps are used to identify serum samples and purple caps are used to identify plasma samples.

B. Labeling Aliquot Vials

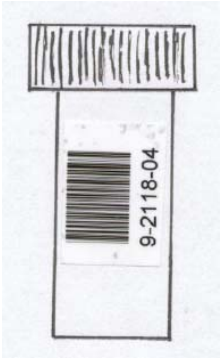
Great care must be taken when labeling aliquot vials. It is critical that the small ID labels applied to the aliquot vials match the participant ID label on the *T1DGC Blood Collection Form*. It also is extremely important that labels are applied firmly and correctly oriented on the vial to minimize labels falling off during shipping and/or storage.

Attach the label to the vial when the vial is at room temperature and leave the cap on the vial when labeling. Apply the label to the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position. That is, the bar-code and readable form of the participant ID on the label should be placed **vertically** rather than horizontally on the vial. The label should not trail off the bottom of the vial or over the cap.

While holding the vial in an upright position, affix the colored portion of the label to the vial first. Wrap the clear tail around the perimeter of the vial. The end of the clear tail should overlap the colored portion of the label. Press **firmly** on the entire label. Verify that all edges of the label adhere to the vial. Freezer tape is not required, but can be used.

When possible, allow newly labeled (empty) vials to set at room temperature for several hours prior to subjecting them to colder temperatures. Labels applied to empty vials 24 – 48 hours in advance of use have better adhesion to the vials.

DIAGRAM FOR LABEL PLACEMENT



LABELING SCHEME

Label type	Use
Large labels	Label each of 3 or 4 tubes collected per participant Label for EDTA tube after plasma aliquoted
Father – blue stripe Mother – pink stripe Proband – purple stripe Affected sibling(s) – green stripe Unaffected sibling(s) – yellow stripe Case – orange stripe Control – gray stripe	
Small vial labels	
Father – blue stripe	Label 5 2-ml cryovials (serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Mother – pink stripe	Label 5 2-ml cryovials (serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Proband – purple stripe	Label 5 2-ml cryovials (autoantibodies and serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Affected sibling(s) – green stripe	Label 5 2-ml cryovials (autoantibodies and serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Unaffected sibling(s) – yellow stripe	Label 5 2-ml cryovials (serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Case – orange stripe	Label 5 2-ml cryovials (autoantibodies and serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)
Control – gray stripe	Label 5 2-ml cryovials (serum storage – red cap) Label 4 2-ml cryovials (plasma storage – purple cap)

C. Aliquoting Samples

Samples for each participant are aliquoted as outlined below.

1. 7.5-ml red top tube: After centrifuging, the serum and the clot should be separated; if not, re-centrifuge for an additional 10 minutes. Unscrew the cap and aliquot serum promptly. For each participant, pipette 0.5-ml aliquots into each of 5 labeled cryovials, using a fresh plastic transfer pipette for each individual. If there is serum remaining after the 5 cryovials have been filled, do not discard it; top off each of the aliquots with additional serum. Re-cap the red top tube and dispose of the capped tube in a biohazard box (*i.e.*, sharps container).

2. 4.9-ml purple top tube: After centrifuging, the plasma should be promptly separated from the cells. Pipette the plasma in 0.5-ml aliquots into each of the 4 labeled cryovials for storage, using a fresh plastic transfer pipette for each individual. If there is plasma remaining after the 4 cryovials have been filled, do not discard it; top off each of the aliquots with additional plasma. **(NOTE: Take special care not to disturb the buffy coat on the cell pack when aliquoting the plasma samples as this will impact the DNA yield adversely.)**

3. The cell pack remaining after plasma is aliquoted from the EDTA tube is shipped to the DNA Repository for DNA extraction. The tube is re-capped and re-labeled with a new, large participant ID label (if needed) following centrifuging and aliquoting processes. The EDTA cell pack is shipped at ambient temperature with the green or yellow top tubes sent daily to the DNA Repository.

ALIQUOTING SCHEME

Tube	Vial(s)	Amount	Use
7.5-ml red top	5 2-ml cryovials (proband, affected sibling, case)	0.5 ml	autoantibodies (1) serum storage (4)
	5 2-ml cryovials (all other family members, control)	0.5 ml	serum storage (5)
4.9-ml purple top	4 2-ml cryovials (each participant)	0.5 ml	plasma storage (4) cell pack for DNA (1)

Note: The cell pack in the EDTA tube is shipped to the DNA Repository for DNA extraction. After all plasma is aliquoted, the EDTA tube is re-capped, re-labeled with a new large participant ID label (if needed), and shipped daily at ambient temperature with the green or yellow top tubes to the DNA Repository.

D. Aliquoting Quality Control Samples

1. The Clinic Coordinator identifies the QC participants and notifies the nurse/technician collecting blood. (Note that participants who return to the clinic for a second blood collection are never selected as a QC participant.) Labels for the QC participants are enclosed in a separate envelope and include large labels for the additional tube to be collected and small vial labels for the QC samples to be aliquoted. For ASP and trio families, the envelopes are labeled with QC-Red "N" or QC-Purple "N", where N is the sequential number of QC IDs within a network. Separate QC label envelopes for the case-control collection are labeled Case QC Red "N" or Control QC Purple "N" where N is the sequential number of QC IDs within a network. Envelopes are opened consecutively within the clinic.

2. Complete Question 7 ("Is participant quality control?") and Question 8 ("Which quality control?") on the *T1DGC Blood Collection Form: Original Collection*. Indicate the type of quality control participant by marking the response "QC-Red" **or** "QC-Purple".

3A. A QC-Red participant must always be a proband, an affected sibling, or a case at least 16 years old (or large for age). For QC-Red participants, label the 7.5-ml red-top tube with a large ID label from the QC envelope and also affix a large QC ID label to page 3 of the *T1DGC Blood Collection Form: Original Collection* (Question 9). Purple-striped QC vial labels (if QC participant is proband), green-striped QC vial labels (if QC participant is affected sibling), or orange-striped QC vial labels (if QC participant is case) are placed on each of 5 2-ml cryovials (1 blind duplicate sample for autoantibodies and 4 storage samples). Only the proband (Participant -03), the first affected sibling (Participant -04), and the case (Participant 7, CAS) should be selected as a QC-red participant. Any additional affected siblings (Participants -07, -08, and -09) should not be selected as a QC-red participant.

3B. After centrifuging the 7.5-ml red top QC tube, transfer 0.5 ml to each of the 5 appropriately labeled 2-ml cryovials, using a fresh plastic transfer pipette for each individual. If there is serum remaining after the 5 cryovials have been filled, do not discard it; top off each of the aliquots with additional serum.

4A. A QC-Purple participant can be any participant in an ASP or trio family, or a control that is at least 16 years old (or large for age). For QC-Purple participants, label one 4.9-ml purple tube with a large label from the QC envelope and also affix a large QC ID label to page 3 of the *T1DGC Blood Collection Form: Original Collection* (Question 9). Place the appropriate color striped label for the selected family member (father-blue; mother-pink; proband-purple; affected sibling-green; unaffected sibling-yellow, or control-gray) on each of 4 2-ml cryovials (plasma storage samples). Additional affected siblings (Participants -07, -08, and -09) should not be selected as a QC-purple participant.

4B. After centrifuging the 4.9-ml purple-top QC tube, transfer 0.5 ml to each of the 4 appropriately labeled 2-ml cryovials, using a fresh plastic transfer pipette for each individual. If there is plasma remaining after the 4 cryovials have been filled, do not discard it; top off each of the aliquots with additional plasma. Take special care not

to disturb the buffy coat on the cell pack as this can impact the DNA yield.

4C. The cell pack in the EDTA tube is shipped to the DNA Repository for DNA extraction. After all plasma is aliquoted, the EDTA tube is re-capped, re-labeled with a new large QC ID label (if needed), and shipped at ambient temperature with the green or yellow top tubes to the DNA Repository.

E. General Comments for Aliquoting Samples

1. The plastic transfer pipettes are graduated in 0.25 ml increments. The approximate capacity is 1 ml. **Two** pipettes should be used for each participant: one to transfer serum and one to transfer plasma.

2. Be careful not to disturb cells when drawing plasma into a pipette. If cells mix with the plasma, re-centrifuge the sample to insure obtaining the maximum amount possible. When using pipettes, avoid drawing red cells into the bulb.

3. Seal or cap all vials immediately; samples should not be allowed to stand open.

VII. RE-COLLECTION OF BLOOD SAMPLES

A. Purpose

In some cases, it may be necessary to contact participants to return to the clinic for a second blood collection. Reasons for a re-collection include: inability to obtain sample(s) during initial clinic visit (including failure to obtain serum samples on a proband, affected sibling or a case); loss of sample(s) due to local freezer failures or shipping errors; failure of the green top (sodium heparin) or yellow top (CPDA) sample to produce a viable cell line for future DNA samples; or low DNA yield from the EDTA cell pack when participant refused cell line. Only one attempt at re-collection should be made for any one participant.

B. Procedure

The *T1DGC Blood Collection Form: Re-collection* should be used to record the second blood collection. The form is formatted and completed in the same way as the

T1DGC Blood Collection Form: Original Collection, with two exceptions. The reason for the re-collection is recorded (Question 2) and there is no section for quality control samples. Participants who return to the clinic for a second collection are not selected for quality control purposes. The procedures for blood collection, sample processing and aliquoting are the same as outlined above for the original collection. There is a separate *T1DGC Blood Collection Form: Re-collection* for the case-control collection.

For a re-collection, labels from the participant's initial clinic visit should be used to label tubes and cryovials. If labels have been discarded or the number of labels is inadequate for the second blood collection, the clinic should contact the Regional Network Center and the Network staff contact the Coordinating Center for additional labels. This should be done well in advance of the participant's re-collection visit.

APPENDIX A

BLOOD COLLECTION, ALIQUOTING AND SAMPLE SHIPMENT:

SUPPLIES LIST

Only Sarstedt supplies will be used for blood collection in the T1DGC. Supplies are ordered from Sarstedt using the master account number provided by the Network Coordinator.

CATALOG NUMBER	DESCRIPTION
01.1613.100	7.5 ML S-MONOVETTE (SODIUM HEPARIN) (CS/500; PKG/50; MIN ORDER: 50)
01.1610.001	8.5 ML S-MONOVETTE (CPDA) (CS/500; PKG/50; MIN ORDER: 50) **UNITED KINGDOM NETWORK USE ONLY**
01.1601.100	7.5 ML S-MONOVETTE (SERUM) (CS/500; PKG/50; MIN ORDER: 50)
04.1931.100	4.9 ML S-MONOVETTE (EDTA PLASMA) (CS/500; PKG/50; MIN ORDER: 50)
85.1638.035	NON-SAFETY MULTIFLY NEEDLE WITH ADAPTER (21G X 0.75"; 200 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)
85.1638.235	SAFETY MULTIFLY NEEDLE WITH ADAPTER (21G X 0.75"; 200 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)
85.1640.035	NON-SAFETY MULTIFLY NEEDLE WITH ADAPTER (23G X 0.75"; 200 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)
85.1640.235	SAFETY MULTIFLY NEEDLE WITH ADAPTER (23G X 0.75"; 200 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)
85.1638.005	NON-SAFETY MULTIFLY NEEDLE WITH ADAPTER (21G X 0.75"; 60 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)

APPENDIX A (CONT.)

BLOOD COLLECTION, ALIQUOTING AND SAMPLE SHIPMENT:

SUPPLIES LIST

CATALOG NUMBER	DESCRIPTION
85.1638.205	SAFETY MULTIFLY NEEDLE WITH ADAPTER (21G X 0.75"; 60 mm tubing) (CS/1000; PKG/100; MIN ORDER: 100)
85.1640.005	NON-SAFETY MULTIFLY NEEDLE WITH ADAPTER (23G X 0.75"; 60 mm tubing) (CS/500; PKG/100; MIN ORDER: 100)
85.1640.205	SAFETY MULTIFLY NEEDLE WITH ADAPTER (23G X 0.75"; 60 mm tubing) (CS/500; PKG/100; MIN ORDER: 100)
85.1373	S-MONOVETTE NEEDLE (21G x 1”) (CS/500; PKG/100; MIN ORDER: 100)
85.1441	S-MONOVETTE NEEDLE (22G x 1”) (CS/500; PKG/100; MIN ORDER: 100)
72.609	2 ML SC MICROTUBE (CRYOVIAL): NO CAP (CS/5000; PKG/500; MIN ORDER: 1000)
65.716.008	SCREW CAP FOR MICROTUBE, VIOLET (CS/10000; PKG/1000; MIN ORDER: 1000)
65.716.003	SCREW CAP FOR MICROTUBE, RED (CS/10000; PKG/1000; MIN ORDER: 1000)
86.1172	TRANSFER PIPETTE (3.5 ML, graduated PE) (CS/5000; PKG/1000; MIN ORDER: 1000)
95.064.997	FIBERBOARD BOXES (to hold 2-ml microtubes) (CS/120; 10/PK; MIN ORDER: 10)

APPENDIX A (CONT.)

BLOOD COLLECTION, ALIQUOTING AND SAMPLE SHIPMENT:

SUPPLIES LIST

SHIPPING CONTAINERS

Asia-Pacific:	Provided by World Courier
European:	Provided by World Courier
North American:	
95.064.928	MAILER SYSTEM (cell line samples) (CS/50; MIN ORDER: 50)
95.064.929	GEL PACKS (cell line samples) (CS/12: MIN ORDER: 12)
96.064.927	SHIPPING CONTAINERS (autoantibody, storage and cell pack samples) (CS/6: MIN ORDER: 6)
96.064.930	ABSORBENT PADS (autoantibody, storage and cell pack samples) (PK/100: MIN ORDER: 100)

NOTE: Gel packs should be kept at room temperature. Do not refrigerate or freeze before use in cell line/cell pack shipments!

United Kingdom:

78.898	POLYPROPYLENE MAILING CONTAINER WITH ABSORBENT LINER (cell lines)
65.679.004	YELLOW CAP FOR MAILING CONTAINER (cell lines)

APPENDIX A (CONT.)

BLOOD COLLECTION, ALIQUOTING AND SAMPLE SHIPMENT:

SARSTEDT CONTACTS

ASIA-PACIFIC NETWORK:

Place all orders to Sarstedt Australia at Sarstedt Australia Pty. Ltd., 16 Park Way Technology Park, South Australia, 5098 (Phone: 0061-8-8349-6555; FAX: 0061-8-8349-4041)

EUROPEAN NETWORK:

Place all orders to Mr. Feuerbach at Sarstedt Germany, Rommeldorfer St., 51588 Numbrecht, Germany (Phone: +49-2293305; FAX: +49-2293305122 or +49-2293305280)

NORTH AMERICAN NETWORK:

Place all orders to Sarstedt USA, 1025 St James Church Road, Newton, NC 28658 (Phone: 800-257-5101; FAX: 828-465-4003)

UNITED KINGDOM NETWORK:
Place all orders to Mr. Feuerbach at Sarstedt Germany, Rommeldorfer St., 51588 Numbrecht, Germany (Phone: +49-2293305; FAX: +49-2293305122 or +49-2293305280)

APPENDIX A (CONT.)

BLOOD COLLECTION, ALIQUOTING AND SAMPLE SHIPMENT:

GENERAL BLOOD COLLECTION SUPPLIES AND EQUIPMENT

Blood Collection Supplies
Alcohol wipes
Emesis basin
Tourniquets
Gauze
Bandaids
Biohazard boxes/bags
Syringes
Freezer (-70^{o}C preferred; -20 ^{o}C acceptable)
Centrifuge (refrigerated)
Test tube racks
Timer
Low temperature freezer tape (to reinforce storage boxes)
Squeeze bottle
Disposable gloves

Shipping Supplies
Dry ice
Biohazard labels
Insulated shipping containers
Gel packs
Ziplock bags

APPENDIX B
BLOOD COLLECTION FORM: ORIGINAL COLLECTION
INSTRUCTIONS FOR COMPLETION

The *T1DGC Blood Collection Form: Original Collection* is to be completed by the nurse or technician at the time of the blood collection. There is a separate form *T1DGC Blood Collection Form: Original Collection (Case-Control)* that should be used for the cases and controls. The form is completed in the same way, following the instructions provided below.

INSTRUCTIONS:

1. Apply a participant ID label to every page and record clinic ID and secondary ID on every page.

2. Record the date of blood collection (Question 1).

3. Before collecting blood, ask the participant, “Do you have any bleeding disorders?” If the participant answers affirmatively, collect blood under the supervision of a physician.

4. Indicate whether any blood was collected (mark “Yes” or “No”), then follow the appropriate skip pattern. When blood is not collected, record the reason (Question 2).

5. Record the time blood was collected, using a 24-hour clock (Question 3).

6. Record the time that the samples BEGIN the centrifuging process, using a 24-hour clock (Question 4).

7. Record the time that the sample vials were placed in the freezer, using a 24-hour clock (Question 5). All serum and plasma samples **must** be frozen within 90 minutes of the blood collection.

8A. Instructions are provided for processing, aliquoting, labeling and storing samples (Question 6).

8B. For the green top or yellow top tubes (Question 6a), mark "Yes" if any tube is collected and mark "No" if no tube is collected. For Question 6b, record the number of green top or yellow top tubes collected (1 or 2) only if "Yes" was marked for Question 6a.

8C. If participant consented to cell line, mark "Yes" for Question 6c. Mark "No" If participant did not consent to cell line and place a pre-printed "DNA Only – No Cell Line" label on the participant's green top or yellow top tube(s) before shipping to DNA Repository.

8D. The serum aliquots for autoantibody analysis and storage are listed with the amount of sample requested (Question 6d). Due to the number of storage vials, each vial is not listed separately on the form. Mark "Yes" if any vials are filled and "No" if none are filled. For Question 6e, record the number of vials filled only if "Yes" was marked for Question 6d.

8E. The plasma aliquots for storage are listed with the amount of sample requested (Question 6f). Due to the number of storage vials, each vial is not listed separately on the form. Mark "Yes" if any storage vials are filled and "No" if none are filled. For Question 6g, record the number of vials filled only if "Yes" was marked for Question 6f.

8F. The cell pack from the 4.9-ml purple top (EDTA) tube is shipped to the DNA Repository for DNA extraction; cell packs are shipped daily at ambient temperature with the cell line samples. Mark "Yes" if the cell pack is available and "No" if it is not.

9A. If the participant has not been selected as a QC participant, mark "No" to the question "Is participant quality control?" (Question 7) and skip to Question 11.

9B. If the participant has been selected as a QC participant, mark "Yes" to the question "Is participant quality control?" (Question 7). Indicate whether the participant was selected as QC-Red or QC-Purple (Question 8). Attach one of the large ID labels from the QC label set in the space provided (Question 9) and complete the aliquoting section (Question 10) in the same manner as above.

9C. **COMPLETE ONLY THE SECTION OF THE FORM THAT PERTAINS TO THE TYPE OF QC PARTICIPANT INDICATED (Question 10).** For example, if the participant was selected as QC-Red, indicate vials that are filled ("Yes") or empty ("No") for the serum samples only. **Do not** enter any information for the plasma samples for QC-Red participants; leave this section blank.

If the participant was selected as QC-Purple, indicate vials that are filled (mark "Yes") or empty (mark "No") for the plasma samples and cell pack only. **Do not** enter any information for the serum samples for QC-Purple participants; leave this section blank.

10. Record the nurse or technician 5-digit ID number on page 5 for the staff member collecting the blood (Question 11) and processing the blood (Question 12). In some clinics, this may be the same individual.

11. Enter the 5-digit ID of the person editing the form (Question 13).

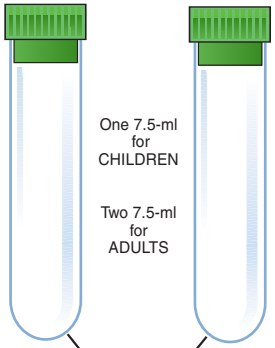
APPENDIX C

BLOOD COLLECTION FLOW CHARTS

TYPE 1 DIABETES GENETICS CONSORTIUM BLOOD FLOW CHART

FILLING PRIORITY: ❶ ❹ ❷ ❸

7.5-ml GREEN (Sodium Heparin) | **7.5-ml GREEN (Sodium Heparin)**



One 7.5-ml for CHILDREN

Two 7.5-ml for ADULTS

Stand at ROOM TEMPERATURE

DO NOT chill, centrifuge, or aliquot

Cell Line

GREEN-top tube

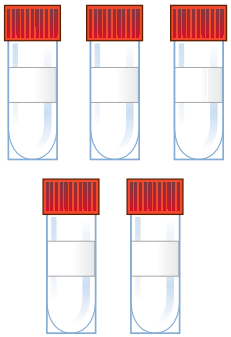
Ship DAILY at ambient temperature → **DNA Repository**

7.5-ml RED (serum) | **4.9-ml PURPLE (EDTA plasma)**

STAND 30 - 60 min. | **ICE 30 - 60 min.**

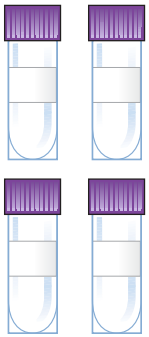
CENTRIFUGE

0.5 ml each *(5 samples)*



Autoantibodies and Serum Storage 2-ml cryovials

0.5 ml each *(4 samples)*



Plasma Storage 2-ml cryovials

Cell Pack

DNA

FREEZE

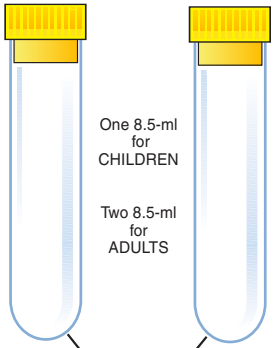
Ship MONTHLY on dry ice → **Autoantibody and Sample Storage Laboratory**

Ship DAILY at ambient temperature → **DNA Repository**

TYPE 1 DIABETES GENETICS CONSORTIUM BLOOD FLOW CHART (UK)

FILLING PRIORITY: ❶ ❹ ❷ ❸

8.5-ml YELLOW (CPDA) **8.5-ml YELLOW (CPDA)**



One 8.5-ml for CHILDREN

Two 8.5-ml for ADULTS

Stand at ROOM TEMPERATURE

DO NOT chill, centrifuge, or aliquot

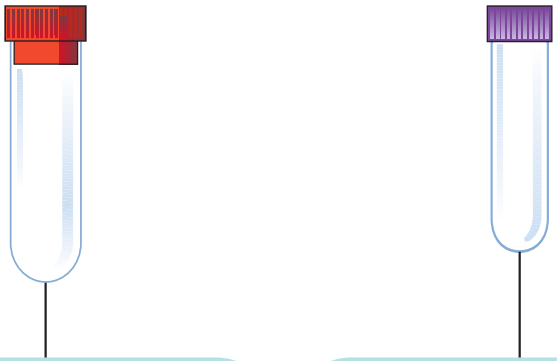
Cell Line

YELLOW-top tube

Ship DAILY at ambient temperature

DNA Repository

7.5-ml RED (serum) **4.9-ml PURPLE (EDTA plasma)**



STAND 30 - 60 min.

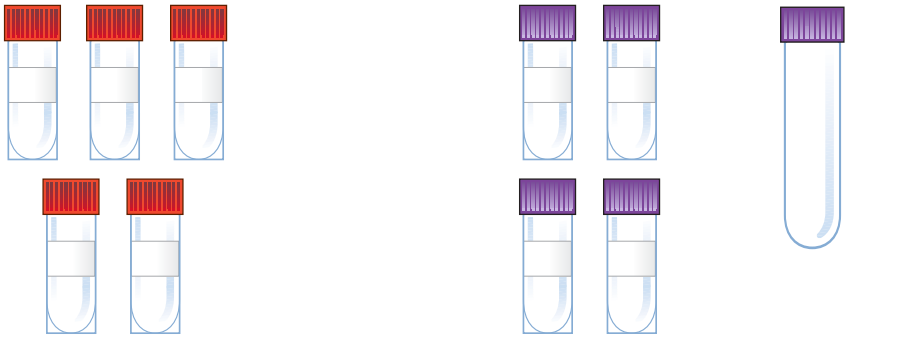
ICE 30 - 60 min.

CENTRIFUGE

0.5 ml each *(5 samples)*

0.5 ml each *(4 samples)*

Cell Pack



Autoantibodies and Serum Storage 2-ml cryovials

Plasma Storage 2-ml cryovials

DNA

FREEZE

Ship DAILY at ambient temperature

Ship MONTHLY on dry ice

Autoantibody and Sample Storage Laboratory

DNA Repository