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CHAPTER 13

SAMPLE HANDLING AND SHIPMENT FOR LIPID AND NON-LIPID TESTS

13.1 Blood Collection and Processing

13.1.1 General Comments

Blood lipid levels can be affected by several factors that occur before or during blood sampling or during storage and transport to the laboratory. Some of these factors include:

1. Posture. It has long been recognized that plasma volume increases, and the concentrations of non-diffusible plasma components decrease, when a standing subject assumes a recumbent position due to redistribution of water between the vascular and extravascular compartments. These fluctuations can be appreciable and can cause changes in lipid and lipoprotein concentrations.
2. Venous occlusion. Prolonged venous occlusion prior to venipuncture can cause appreciable increases in the apparent concentration of non-diffusible components. Serum cholesterol concentrations were found to increase an average of 10-15% after a 5 minute period of occlusion. Errors of this magnitude should not normally occur, since the tourniquet is usually removed within 30-60 seconds.
3. Fasting. Recent food intake exerts little if any effect on plasma total cholesterol concentration. Plasma triglycerides, however, increase in postprandial plasma. This is due to the appearance of chylomicrons in the circulation after a fat-containing meal. Chylomicron clearance normally requires a few hours, and no chylomicrons should be present after a 12

hour fast. In this study, subjects will have been asked to fast for 12 hours before venipuncture.

13.1.2 Blood Drawing

13.1.2.1 Introduction

In this section, we present detailed venipuncture procedures. We realize you may be experienced in venipuncture and do not mean to indicate otherwise by presenting this material in such detail. We have done so to help insure standardization.

13.1.2.2 Set Up of Medical Supplies and Equipment

Prior to meeting with the participant, the nurse/phlebotomist will assemble the venipuncture equipment for each participant, including:

1. Vacutainer holders.
2. 20G needles.
3. Set up the required number of vacutainer tubes (see Section 13.2.5 below); make sure that the tube stoppers have not been lubricated with glycerol, which interferes with triglyceride assays. Use tubes with silicone-lubricated stoppers.
4. Tourniquet.
5. Alcohol wipes, Betadine wipes.
6. 2 x 2 gauze pads.
7. Band aids.
8. Disposable gloves.

13.1.2.3 Preparing the Patient for Venipuncture

Explain the venipuncture procedure to the participant. The participant may be apprehensive about this procedure. A calm and reassuring explanation of the procedure can help the participant

overcome his/her apprehension and give him/her confidence in the person performing the venipuncture. Answer the participant's questions concerning the procedure as clearly and concisely as possible. You might tell him/her that you're going to take the blood in separate tubes, so you'll be switching tubes but only doing the venipuncture itself once. Also, you might suggest he/she not watch. Remind him/her there is a possibility of slight bruising at the puncture site.

A common fear that participant may have when blood is being drawn is that a great amount of blood is being taken. For these tests, up to about 30 ml of blood will be taken. This can be translated into terms better understood by the participant. Since he/she probably understands tablespoons better than milliliters, 30 ml is equal to about three tablespoons. If the participant expresses fear about whether he/she can bear the loss of this amount of blood, you might reassure him/her by telling him/her that his/her body is manufacturing and replacing blood daily, whether he/she loses any blood or not.

If necessary, have the participant remove any bulky, heavy, or constricting clothing that may interfere with accessibility to the arm for the procedure.

13.1.2.4 Venipuncture Procedure

Steps are as follows:

1. Instruct the participant to lie down on the bed/litter provided. Since postural changes can affect blood cholesterol concentrations (see above), the venipuncture procedure should be

performed within no more than 5 minutes. If it takes longer, have the participant get up and move around for 10 minutes before trying again. Never attempt a venipuncture on a standing participant. Participants sometimes faint after a venipuncture, and a standing participant might suddenly collapse, causing an unnecessary and preventable injury.

2. Instruct the participant to extend his/her arm, palm up, and straight at the elbow.
   a. Be sure the participant is positioned so that the veins to be used are readily accessible and that you are able to work in a comfortable position.
   b. The participant's arm can be supported on a roll of towels to increase comfort.
3. Place your venipuncture equipment where it is readily available, but is not in danger of being upset by the participant. Keep extra equipment and tubes within easy reach.
4. Thoroughly wash your hands before and after each venipuncture and don a pair of disposable gloves.
5. First, inspect the arm you plan to use for the venipuncture. You may be able to see some veins. Use the following characteristics to differentiate a vein from an artery:
   a. Veins are usually superficial, while arteries are deeply placed and are protected by surrounding musculature.
   b. Arteries are more elastic and have a thick, rough wall.
   c. Veins do not pulsate; arteries do pulsate.
   d. Venous blood is dark red in color; arterial blood is a bright red color.
6. The veins of choice for the venipuncture procedure are those located in the antecubital area. Do not draw blood from any arm

with an arterial access (i.e., fistula, shunt), with a rash, or open sores. In addition, any arm that is swollen or edematous should not be used for venipuncture.

7. Apply the tourniquet directly above the elbow. The tourniquet should be applied with sufficient pressure to prevent venous return.
   a. To aid in participant comfort, two facial tissues may be placed under the tourniquet.
   b. Avoid putting the tourniquet on too tightly.
8. Select a vein that is palpable and well-fixed to surrounding tissue. Palpate even when the vein can be seen. If the veins do not distend rather quickly, the following techniques may be used:
   a. Have the participant open and close his/her hand several times.
   b. Massage the arm from wrist to elbow, which forces blood into the veins.
   c. Tap the area sharply with the index and second finger two or three times: this causes the veins to dilate.
   d. The arm to be used for venipuncture may be hung dependently at the participant's side without a tourniquet. This will allow the veins to fill with blood to their capacity.
   e. Examine the participant's other arm. Sometimes the veins in one are small while those in the other arm are larger.
9. If the tourniquet has been applied for more than one minute while you search for a vein, release the tourniquet for one to two minutes. Prolonged obstruction of blood flow by the tourniquet changes some test results.

10. Cleanse the area with the alcohol wipe. Hold the alcohol wipe with two fingers on one side of it, so that only the other side of the wipe touches the area of the puncture site. Cleanse the area using a circular motion; begin with a narrow radius and move outward so as not to cross over the area already cleansed. Repeat with a second alcohol wipe. Dry the cleansed area using a dry sterile 2 x 2 gauze pad or cotton ball. The area should be completely dry before the venipuncture to reduce pain and to eliminate the possibility of carrying alcohol into the blood sample.
11. After the puncture site is selected and cleansed, attach a 20G needle to the vacutainer holder and insert the first red top tube up to the line on the adapter, so it is secure and will not fall out. TAKE CARE NOT TO BREAK THE VACUUM ON THE TUBE UNTIL THE NEEDLE IS SECURELY IN THE VEIN.
12. Fix the selected vein about one inch below the proposed site of puncture by pulling the skin taut with the thumb of the free hand.
13. Point the needle, bevel up, in the direction of the course of the vein, at a 45 degree angle to the skin surface, and about one-half inch below the proposed puncture site. Be sure the needle bevel is up so there is less trauma to the skin and vein.
14. Insert the needle, bevel up, at a 45 degree angle so that it enters the skin first and then the vein, push the first red top tube into the adapter.
    a. If the needle is in the vein, blood will flow freely into the tube.

b. If no blood enters the tube, remove the needle and switch to the other arm using a new sterile needle. If for some reason you must use the same arm for the second try, wait ten minutes before beginning the procedure again, using a new sterile needle.

c. Two attempts for a successful venipuncture are allowed in children; three attempts can be made in adults.

15. After the tube is full, the blood will stop flowing into the chamber. Pull the tube off the adapter and replace it with the next tube. Push the tube completely into the adapter breaking the vacuum as before. After the tube is full, the blood will stop flowing into the chamber. Do not release the tourniquet until the last vacutainer is filling. Each tube should be held lower than the venipuncture site to prevent backflow through the tubing.

16. Place a sterile 2 x 2 gauze pad over the puncture site with one hand and release the tourniquet with the other hand.

a. Withdraw the needle with the adapter and tube still attached with a slow but firm motion. A slow withdrawal is less painful for the participant and decreases the chance of cutting the vein.

b. When the needle is out of the arm, have the participant hold the gauze over the puncture site for two to four minutes with mild pressure. Tell the participant not to blot the puncture site (i.e., pick the gauze up and down), but apply constant pressure.

c. Firm pressure over the puncture site will prevent leakage of blood into surrounding tissues with subsequent hematoma development.

d. Flexing the arm may not prevent a hematoma, since the vein can slip to the side of the area where pressure is applied.

17. Inspect the puncture site. When the bleeding has stopped, cover the site with a band aid.

a. Instruct the participant to remove the band aid after one to two hours.

b. Do not use a band aid on any participant who states he/she has an allergy to adhesive.

18. Information on possible complications of venipuncture is presented below.

13.1.3 Complications of Venipuncture

13.1.3.1 Hematomas

Hematomas are the most common complication of venipuncture. They are masses produced by coagulation of extravasated blood in a tissue or cavity. Hematomas may result from through-and-through puncture to the vein or from incomplete insertion of the needle into the lumen of the vein, allowing the blood to leak into the tissue by way of the bevel of the needle. In the latter case, correction may be made by advancing the needle into the vein. At first sign of uncontrolled bleeding, the tourniquet should be released and the needle withdrawn. Mild pressure to the puncture site should be applied immediately.

Hematomas also result from the application of the tourniquet after an unsuccessful attempt has been made to draw blood. They most frequently result from insufficient time spent in applying the pressure, from failure to apply pressure, and from the BAD HABIT OF FLEXING THE ARM TO STOP BLEEDING.

Once the venipuncture is complete, the participant should be instructed to apply mild pressure to the puncture site. Constant pressure should always be maintained until the bleeding stops. Pressure should be applied with dry, sterile gauze; a wet sponge encourages bleeding. Band aids do not take the place of pressure and, if used, are not applied until the bleeding stops.

Arms covered with ecchymoses (escape of blood into the tissues, producing a large and blotchy area of superficial discoloration; bruises) demonstrate poor technique or haphazard manner. Proper technique must be employed at all times to prevent unnecessary hematomas.

13.1.3.2 Syncope (Fainting)

1. Sudden loss of strength or temporary loss of consciousness.
2. Caused by decreased blood flow to the brain.
3. To prevent injury of any participant who might faint, always perform the venipuncture when the patient is in a horizontal, relaxed position.
4. Warning signs:
    a. The participant may become pale and begin to perspire heavily.
    b. The participant may feel dizzy and hot, and begin to pant (hyperventilate).
    c. The participant may feel nauseated.
5. When the participant has any of the above signs:
    a. Instruct the participant not to watch the procedure.
    b. Have the seated participant put his/her head down between his/her knees.

c. Have the participant take slow, deep breaths.

d. Have emesis basin within reach.

e. Keep talking to the participant in a calm, reassuring manner.

6. If the participant faints:

a. Gently, ease the participant to a lying position.

b. Elevate his/her feet.

c. Check blood pressure and radial pulse.

d. Use ammonia ampules.

e. After the participant regains consciousness, give him/her fluids, i.e., orange juice or water.

f. Again, have emesis basin within reach should the participant feel nauseated.

13.1.3.3 Continued Bleeding

Participants receiving certain drug therapies or participants with bleeding disorders may continue to bleed after the venipuncture. To prevent bleeding, it may be necessary to apply pressure to the puncture site for an extended period of time. Apply a pressure dressing to the puncture site after the bleeding stops.

If the participant continues to bleed after ten minutes, take him/her to the nearest emergency room and wait until he/she is released.

13.1.3.4 Thrombosis

This is the formation of blood clots (thrombi) inside a blood vessel or inside the chambers of the heart. Thrombi can occur as a result of venipuncture when the endothelial lining of the vein is injured. A thrombosed vein should not be used for venipuncture. A thrombosed vein can be detected by palpation prior to the venipuncture. The

vein with a thrombosis lacks resilience, feels hard and cord-like, and rolls easily.

Only the veins in the arms will be used for the venipuncture procedure. Veins in the lower extremities have deep connections and may have poor circulation, which leads to the formation of thrombi. To prevent thrombosis, subsequent venipunctures should be performed at sites proximal to previous puncture sites.

13.1.3.5 Sclerosis

This condition is an induration or hardening of blood vessels. It can occur as a result of inflammation, excessive venipuncture, and poor technique. A vein that feels hard when palpated should not be used for venipuncture. Prevention of sclerosis can be accomplished by the skillful performance of venipuncture technique.

13.1.3.6 Embolus

An embolism is transfer of a mass, a blood clot or object within the vascular system, from its point of origin or entrance to a distant site, causing an obstruction of blood flow. The embolus is most often a blood clot, but it may be a fat globule, an air bubble, a piece of tissue, or a clump of bacteria. Embolisms are usually fatal, and can be prevented by performing the venipuncture procedure using skillful technique.

13.1.3.7 Accidental Needle Stick or Contamination of Open Wound (of Venipuncture Technician)

This can occur as a result of careless technique and improper disposal of used needles and blood drawing equipment.

To prevent puncture wounds, wash hands thoroughly between participants and before and after handling blood samples. Observe aseptic blood drawing technique. Dispose of used needles in needle boxes; NEVER IN A WASTEBASKET. Discard other blood drawing equipment in the proper waste receptacles.

If an accidental needle stick or contamination occurs, go to the nearest hospital for instructions regarding the need for gamma globulin. Contact your supervisor as soon as possible.

13.1.3.8 Drawing Blood From Contaminated Respondent

As with all blood drawing, a sterile technique should be used. Used needles should be disposed of in a "Sharps bottle" or similar container. Do not discard needles in the trash or wrapped in soft wrapping such as tape, paper, etc. Isolate and dispose of non-sharp blood drawing supplies as appropriate for contaminated biological waste.

Blood should be marked as "contaminated," "infectious," "possible infectious," or with the specific infectious process, if known. The blood should then be double wrapped in biohazard bags, and disposed of as contaminated biological waste.

Should anyone be accidentally stuck by the contaminated needles, he/she should report this to his/her supervisor and go to the nearest emergency room for a gamma globulin injection.

13.1.4 Emergency Procedures

Should an emergency arise, call the nearest Emergency and Ambulance Rescue Team, and have them send an ambulance to the office. As soon

as the Rescue Team arrives, call the nearest hospital and tell them the participant is on his/her way.

You should also have a "crash kit" in each office site. This kit is not intended to use, but could be used by the Rescue Team if needed or if a physician from a nearby emergency room instructed the nurse/phlebotomist in the office to do so.

13.1.5 Samples to Be Collected

The following blood should be drawn for the lipid, non-lipid, and hormone tests in DISC I. These apply to the children unless otherwise indicated.

SV1	1 red top tube, 15 ml (for lipids, lipoproteins, apoproteins, permanent storage)
SV2	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (non-lipid tests, hormones) or 1 red top tube, 10 ml (non-lipid tests) for clinics not participating in hormone ancillary study
	1 royal blue top, 7 ml (Zn^{+2}/Cu^{+2}, non-lipid tests)
	1 purple top, 2 ml (CBC, red cell folate)
BV0 (adults)	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins for Cohort 1 only)
IV01 (interv. adults)	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins for Cohort 2+)
6 mos	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins for Cohort 1 only)
12 mos	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (non-lipid tests, hormones)
	1 royal blue top, 7 ml (Zn^{+2}/Cu^{+2}, non-lipid tests)
	1 purple top, 2 ml (CBC, red cell folate)

36 mos	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (non-lipid tests, hormones)
	1 royal blue top, 7 ml (Zn^{+2}/Cu^{+2}, non-lipid tests)
	1 purple top, 2 ml (CBC, red cell folate)
36 mos (adults)	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins)
37 mos	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins)
	1 red top tube, 15 ml (hormones)
48 mos (Cohort 1 only)	1 red top tube, 15 ml (lipids, lipoproteins, apoproteins)
	1 red top tube, 15 ml (hormones)

The following blood should be drawn for lipid, non-lipid, hormone tests, and DNA analyses in DISC II. These apply to children only. No blood will be drawn on adults in DISC II. Blood will not be drawn in Years 6 and 8.

48 mos	No blood will be drawn on Cohort 2 and higher participants.
Year 5	2 red top tubes, 15 ml and 10 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (hormones)
Year 7	2 red top tubes, 15 ml and 10 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (hormones)
	1 purple top, 5 ml (DNA Ancillary Study) One-time collection at an annual or final visit.
Year 9	2 red top tubes, 15 ml and 10 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (hormones)
	1 purple top tube, 5 ml whole blood (DNA analyses) One-time collection at an annual or final visit.

Final Visit 1	2 red top tubes, 15 ml and 15 ml (lipids, lipoproteins, apoproteins, non-lipids, and permanent storage)
	1 red top tube, 15 ml (hormones)
	1 royal blue top, 7 ml (Zn^{+2}/Cu^{+2})
	1 purple top, 2 ml (CBC, red cell folate)
	1 purple top, 5 ml (DNA analyses) One-time collection at an annual or final visit.
Final Visit 2	2 red top tubes, 15 ml and 10 ml (lipids, lipoproteins, apoproteins, permanent storage)
	1 red top tube, 15 ml (hormones)
	1 purple top, 5 ml (DNA analyses) One-time collection at an annual or final visit.

Note: The royal blue top tubes will be supplied to the Clinical Centers by the CDC laboratory, which will procure and evaluate the tubes to insure that they are trace metal free before they are distributed. Until they become available, (about the first part of December, 1987), the Clinical Centers will purchase these tubes directly (see Supplies section below). A random sample of five locally procured tubes from each center should be sent to the Central Laboratory, which will forward them to the CDC laboratory for similar evaluation at some future date. There is no need to wait for the results of this evaluation before using the locally procured tubes, however.

The tubes should be drawn in the following order:

First: 15 ml red top

Second: 10 ml red top

Third: 7 ml royal blue top

Fourth: 2 ml purple top

Fifth: 15 ml red top

Sixth: 5 ml purple top

(By drawing the royal blue top tube third, the needle will have been first flushed with the 10 ml of blood drawn into the second tube.)

13.1.6 Certification and Recertification for Phlebotomist

13.1.6.1 Requirements for Certification

1. Read and be familiar with Section 13.1 of this chapter.
2. Complete training with the local Master Trainer.
3. Section A of the Local Laboratory Procedures Checklist (Exhibit 13.1) should be completed by Master Trainer.

13.1.6.2 Requirements for Recertification (Annually)

1. Draw blood from 10 participants according to DISC Protocol in previous 3 months.
2. Section A of the Local Laboratory Procedures Checklist should be completed by Master Trainer.

13.2 Sample Handling and Shipment

13.2.1 Serum Preparation

1. After the sample has been drawn into red top or royal blue top glass collection tubes, allow the blood to stand for 45 min at room temperature to allow complete clotting and clot retraction. A shorter period may result in incomplete clotting and secondary clots may form later. During the clotting period leave the collection tube sealed.
2. Centrifuge the samples at 1500 x g for 20 min at 40°C. If a refrigerated centrifuge is not available, a room temperature centrifuge can be used.
3. Red top tubes. Remove the tube stopper and gently work a disposable applicator stick around the walls of the tube ("rim the tube"). Be careful not to cause hemolysis during this step.

4. Royal blue top tube. The royal blue top tube is a trace metal-free tube that is used for the analysis of zinc and copper. This sample is allowed to clot as described for the red top tubes above. Do not unseal the tube or run the clot, however. The tube should remain sealed and centrifuged as above. Care must be taken that the sample does not come into contact with any surfaces that may contain Zn^{+2}/Cu^{+2}. After centrifugation, the serum is transferred into the special polypropylene vial provided by CDC (see below) using the one piece trace metal-free polypropylene pipet provided by CDC. Do not use a transfer pipet or other device that must contact the serum. After the clean sample has been removed for the Zn^{+2}/Cu^{+2} analyses, the remaining serum can be used as described above for the red top tubes. Note: The royal blue top tube stoppers are lubricated with glycerol which interferes with the triglyceride assay. Therefore, do not mix the serum from the royal blue top tube with the serum from the red top tubes until the aliquots for lipid analysis have been removed from the red top tube serum.

5. If blood is collected into more than one red top blood collection tube, transfer the serum from all red top tubes into a single temporary storage container, such as a 20 ml scintillation counting vial equipped with a polyethylene lined screw cap. Seal the vial and invert several times to mix the sample gently but thoroughly. Do not use the extra serum from the royal blue top tube unless necessary (see Section 13.3.2, Item 5, Sampling Priorities, below). Place the temporary storage container in a refrigerator and store at 2-4°C until the appropriate aliquots are prepared and frozen (see below).

13.2.2 Preparation of Sample Aliquots for Lipoprotein Analysis in DISC I

Tables 1 and 2 give the aliquots that should be prepared for the analysis of lipids, lipoproteins and apoproteins in DISC I.

Table 1

Aliquots for Lipids, Lipoproteins, Apoproteins by Visit
Children in DISC I

TEST	SV1	SV2	MN06*	MN12	MN36	MN37	MN48*
TC/TG/HDL	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
apoAI/apoB	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
permanent storage	0.5 ml (x 2)	0.5 ml (x 2)	---	0.5 ml (x 2)	0.5 ml (x 2)	---	---

*Cohort 1 only

Table 2

Aliquots for Lipids, Lipoproteins, Apoproteins by Visit
Adults in DISC I

TEST	IV01**	MN36
HDL	3 ml	3 ml
TC/TG	4 ml	4 ml
apoAI/apoB	0.5 ml	0.5 ml

**Intervention group only.

Note: The decision has been made to collect serum from the parents of all children in both the control and intervention groups at 36 months, and for intervention group parents only at the first intervention visit. These samples will be stored at -70°C at the Central Laboratory and analyzed at the end of the study. It will be necessary, however, to prepare the HDL-containing fraction as soon as possible. For this reason it will be necessary to send two aliquots for lipid/lipoprotein analysis. The 3 ml aliquots will be thawed and the HDL-containing fraction will be prepared when the samples are

received. The HDL-containing fraction will then be stored at -70°C with the 4 ml aliquot and the apoprotein aliquot, and all of the analyses will be performed at the end of the study.

13.2.3 Preparation of Sample Aliquots for Lipoprotein Analysis in DISC II

Table 3 gives the aliquots that should be prepared for the analysis of lipids, lipoproteins, and apoproteins in DISC II for children.

Table 3

Aliquots for Lipids, Lipoproteins, Apoproteins by Visit
Children in DISC II

TEST	MN48†	YR05	YR06	YR07	YR08	YR09	FV01	FV02
TC/TG/HDL	---	5 ml	---	5 ml	---	5 ml	5 ml	5 ml
apoAI/apoB	---	0.5 ml	---	0.5 ml	---	0.5 ml	0.5 ml	0.5 ml
Permanent storage	---	0.5 ml (x 2)	---	0.5 ml (x 2)	---	0.5 ml (x 2)	0.5 ml (x 2)	---

†Cohorts 2 and higher

13.2.4 Sample Preparation for Non-Lipid Tests in DISC I

The following aliquots should be prepared for the non-lipid tests that will be performed at Johns Hopkins (Table 4) for children in DISC I.

Table 4

JHU Aliquots for Non-Lipid Tests by Visit
Children in DISC I

TEST	SV1	SV2	MN06	MN12	MN36	MN37	MN48*
Chem panel	---	1 ml	---	---	1 ml	---	---
T4, ferritin	---	1 ml	---	---	---	---	---
Albumin	---	---	---	1 ml	---	---	---

*Cohort 1 only.

The following aliquots should be prepared for the non-lipid tests that will be performed at CDC (Table 5) in DISC I.

Table 5

CDC Aliquots for Non-Lipid Tests by Visit
Children in DISC I

TEST	SV1	SV2	MN06	MN12	MN36	MN37	MN48*
Zn^{+2}/Cu^{+2}	---	2 ml	---	2 ml	2 ml	---	---
Retinol, Toc., Carot.	---	1 ml	---	1 ml	1 ml	---	---
Ferritin	---	1 ml	---	1 ml	1 ml	---	---
Linoleate/ Oleate	---	1 ml	---	---	---	---	---
Red Cell Folate (whole blood)	---	0.1 ml	---	0.1 ml	0.1 ml	---	---

*Cohort 1 only.

All of the non-lipid tests will be performed in serum except red cell folate. This analysis is performed in a red cell hemolysate that is to be prepared as follows:

Preparation of ascorbic acid solution. Bottles containing dry ascorbic acid will be sent to the Clinical Centers from the Central Laboratory. Keep the bottles capped and dry. Reconstitute the dry ascorbic acid with the amount of distilled water indicated in the instructions that will accompany the bottles. The final concentration of ascorbic acid should be 1% by weight. Reconstitute immediately before use and store in a refrigerator. At the end of the day, discard any unused ascorbic acid solution and reconstitute a fresh bottle for use each day.

Preparation of Hemolysate

With the 1.0 ml repeating pipet (see Section 13.6, Supplies, below) place 1.0 ml of 1% (wt/vol) ascorbic acid solution into 6 ml polypropylene vial. Set up enough vials for the day's activities.

Mix the blood in the 2 ml purple top tube gently to suspend the red cells evenly. With the 0.1 ml repeating pipet, remove a 0.10 ml aliquot of whole blood from the purple top tube and add it to the ascorbic acid. Cap the vial and mix well. (Use a vortex mixer if available. Otherwise invert the sample eight times.)

The sample is then stored at -20°C until it is shipped. Note: the remaining whole blood in the purple top tube is used for a complete blood count (CBC), which will be determined locally at the Clinical Center and will not be sent to the Central Laboratory for analysis.

13.2.5 Sample Preparation for Non-Lipid Tests in DISC II

The following aliquots should be prepared for the non-lipid tests that will be performed at Johns Hopkins (Table 6) in DISC II.

Table 6

JHU Aliquots for Non-Lipid Tests by Visit
Children in DISC II

TEST	MN48†	YR05	YR06	YR07	YR08	YR09	FV01	FV02
Chem panel	---	---	---	---	---	---	5 ml	5 ml
Ferritin	---	---	---	---	---	---	0.5 ml	0.5 ml

†Cohorts 2 and higher

The following aliquots should be prepared for the non-lipid tests that will be performed at CDC (Table 7) in DISC II.

Table 7

CDC Aliquots for Non-Lipid Tests by Visit
Children in DISC II

TEST	MN48†	YR05	YR06	YR07	YR08	YR09	FV01	FV02
Zn^{+2}/Cu^{+2}	---	---	---	---	---	---	2 ml	---
Retinol, Toc., Carot.	---	---	---	---	---	---	1 ml	---
Ferritin	---	---	---	---	---	---	1 ml	---
Red Cell Folate (whole blood)	---	---	---	---	---	---	0.1 ml	---

†Cohorts 2 and higher.

13.2.6 Sample Preparation for Hormone Tests

The following aliquots of serum should be prepared for the hormone analyses on children that will be performed under the direction of the National Cancer Institute (NCI) (Table 8) in DISC I.

Table 8 (Hormone Study)

Hormone Aliquots by Visit
Children in DISC I

TEST	SV1	SV2	MN12	MN36	MN37	MN48*
Steroids	---	1 ml	1 ml	1 ml	1 ml	1 ml
Bioavailable Fractions	---	1 ml	1 ml	1 ml	1 ml	1 ml
SHBG	---	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml

*Cohort 1 only.

The following aliquots of serum on children should be prepared for hormone analyses in DISC II (Table 9).

Table 9 (Hormone Study)

Hormone Aliquots by Visit
Children in DISC II

TEST	MN48†	YR05	YR06	YR07	YR08	YR09	FV01	FV02
Steroids	---	2 ml	---	---	---	---	---	---
Bioavailable Fractions	---	2 ml	---	---	---	---	---	---
SHBG	---	1 ml	---	---	---	---	---	---
All tests	---	---	---	5 ml	---	5 ml	5 ml	5 ml

†Cohorts 2 and higher.

Please note that hormone aliquots for DISC II are double the amount collected in DISC I. Excess serum should not be discarded, but should be added to hormone study aliquots. All samples should be sent to Johns Hopkins for storage.

13.2.7 Sample Preparation for DNA Analyses in DISC II

A sample of four whole blood spots on filter paper should be taken from the one-time collection (at an annual or final visit) of 5 ml of whole blood in a 5 ml purple-top tube. The DNA Ancillary Study Protocol is appended as Exhibit 13.2.

13.2.8 Certification and Recertification for Sample Preparation

13.2.8.1 Requirements for Certification

1. Read and be familiar with Section 13.2 of this chapter.
2. Complete training with local Master Trainer.
3. Section B of the Local Laboratory Procedures Checklist (Exhibit 13.1) should be completed by the Master Trainer.

13.2.8.2 Requirements for Recertification

1. Prepare the specimens from 10 participants according to DISC Protocol in previous 3 months.
2. Section B of the Local Laboratory Procedures Checklist (Exhibit 13.1) should be completed by the Master Trainer.

13.3 Sample Storage and Shipment for DISC I and II

13.3.1 Packaging

The samples will be shipped to the Central Laboratory, which will then analyze the lipids, lipoproteins and apoproteins, store the hormone and DNA blood spot samples, and forward the non-lipid test samples to the appropriate laboratories for analysis.

The samples will be packaged as shown in Table 10.

Table 10

Type of test	Type of sample	Type of shipping container
CHILDREN		
Lipid/lipoprotein	5 ml serum (TC/TG/HDL)	5 ml serum bottle
Apoproteins	0.5 ml serum (apoAI\apoB)	2 ml serum bottle
Permanent Storage	0.5 ml	2 ml serum bottle
Non-lipid tests at Johns Hopkins	1 ml serum (chem panel)	2 ml serum bottle
	1 ml serum (T4/ferritin)	2 ml serum bottle
	1 ml serum (albumin)	2 ml serum bottle
Non-lipid tests at CDC	2 ml serum from royal blue top tube (Zn^{+2},Cu^{+2})	6 ml polypropylene vial*
	1 ml serum (carotinoids, tocopherol, retinol)	6 ml polypropylene vial
	1 ml serum (ferritin)	6 ml polypropylene vial
	1 ml serum (linoleate/oleate)	6 ml polypropylene vial
	red cell hemolysate	6 ml polypropylene vial
Hormone analyses for Hormone Study		
DISC I	1 ml serum (steroids)	2 ml serum bottle
	1 ml serum (bioavailable fractions)	2 ml serum bottle
	0.5 ml serum (SHBG)	2 ml serum bottle
DISC II (Year 5)	2 ml serum (steroids)	5 ml serum bottle
	2 ml serum (bioavailable fractions)	5 ml serum bottle
	1 ml serum (SHBG)	2 ml serum bottle

*Supplied by CDC.

Table 10 (Continued)

Type of test	Type of sample	Type of shipping container
CHILDREN		
DISC II (Year 7, Year 9, and Final Visits)	5 ml serum (steroids, bioavailable fractions and SHBG)	10 ml serum bottle
DNA analyses for DNA Ancillary Study (DISC II one-time sample)	4 whole blood spots on filter paper: ApoA-I Promoter Genotype ApoE Genotype ApoA-IV Genotype	pouch and manilla envelope
ADULTS	3 ml serum (HDL)	5 ml serum bottle
	4 ml serum (TC/TG/HDL repeat)	5 ml serum bottle
	0.5 ml serum (apoproteins)	2 ml serum bottle

The serum bottles are sealed with the rubber stoppers and aluminum seals. The polypropylene vials are sealed with the screw caps provided.

13.3.2 Procedure

1. Samples for lipid, lipoprotein and apoprotein analyses: Assemble the required number of 5 ml and 2 ml bottles. Affix the pressure sensitive sample identification labels to each. The pressure sensitive labels should be further fixed in place with "Scotch Magic Tape." This is necessary because the pressure sensitive labels have a tendency to come off the vials, particularly at low temperature.
2. Transfer the required amount of serum into each storage vial, using a disposable transfer pipet. These are available in jumbo size for handling milliliter quantities of sample. Make sure that a sufficient volume of serum is transferred to each vial. This can be done without actually measuring the sample volumes by using dummy vials containing the appropriate volumes of water as guides for comparison.
3. Cap each vial with the rubber stopper and place an aluminum seal on the stopper. Using the cap crimper, seal the aluminum cap onto the vial. All of the vials containing aliquots from a single participant are placed into a 2-1/4" x 5" plastic bag which is closed and sealed with tape. Prepare the sample log sheets listing the sample ID, date samples were drawn, shipment date and identification of individual who prepared the samples. Place a copy of the form along with the small plastic bags containing the aliquots from participants listed on the log sheet into the 5" x 8" plastic bag. This is to facilitate

handling; all aliquots from a single participant are kept together. The sealed samples are then stored in a -20°C freezer until shipped.

4. The samples for non-lipid tests are grouped similarly placing all the samples from a single participant into a small plastic bag, and placing these packages along with non-lipid test log sheets into the larger plastic bag. The samples are stored at -20°C until they are shipped.

5. The samples for hormone tests should be grouped similarly to those for other tests. Place all the samples from a single participant into a small plastic bag, and put these packages, along with the hormone test log sheet (Form 55), into the larger plastic bag. The samples should be stored at -20°C until they are shipped to Johns Hopkins for storage.

6. Sampling Priorities. In the event sufficient sample cannot be obtained for all of the analyses, the following priorities should be observed when preparing the aliquots.

1st:	Tot chol, trig, HDL	(5 ml)
2nd:	Apoproteins	(0.5 ml)
3rd:	Chem panel	(1 ml)
4th:	T4/ferritin	(1 ml)
5th:	Ferritin (for CDC)	(1 ml)
6th:	Red Cell folate, CBC	(2 ml whole blood)
7th:	Zn^{+2}/Cu^{+2}	(2 ml)
8th:	Retinol, tocoph, carotenoids	(1 ml)
9th:	Linoleate/Oleate	(1 ml)
10th:	Permanent storage	(2 x 0.5 ml)
11th:	Steroids	(1 or 2 ml) Hormone Study

12th:	BioAvailable fractions	(1 or 2 ml) Hormone Study
13th:	SHBG	(0.5 or 1 ml) Hormone Study
14th:	DNA Blood Spot Sample	4 whole blood spots (about 20 drops) DNA Ancillary Study

Note: Since care must be taken to prevent contamination of the Zn^{+2}/Cu^{+2} sample, the 2 ml aliquot for these analyses should be prepared first when the royal blue top tube is opened. In general the royal blue top tube should only be used for the trace metals. If there is sufficient serum for the higher priority tests, the trace metal sample can be used and can be handled in the same way as the samples from the red top tubes. As mentioned above, the serum from the royal blue top tube SHOULD NOT BE USED FOR TRIGLYCERIDE ANALYSIS. The stoppers for the royal blue top tubes are lubricated with glycerol, which interferes with triglyceride assays.

7. Caution in handling samples for Zn^{+2}/Cu^{+2} analysis. Trace metal contamination can occur from dust particles in the air. For this reason, neither the serum tubes nor the polypropylene containers used for these metals should be left uncovered for longer than necessary. Do not unstopper the royal blue top tube or remove the cap from the polypropylene vial until immediately before the serum is to be transferred. Then cap the vial immediately.

13.4 Shipment

At the time of shipment, the vials are removed from the freezer and arranged in batches according to the sample log sheets. If it has not yet been done, each batch of samples is placed into a 5" x 8"

plastic bag, along with the log sheet for that batch of samples. The samples are then placed in a styrofoam shipping container along with enough dry ice to keep the samples frozen for at least 48 hours.

Address the shipping container to:

Ms. Kat Lovejoy
Lipoprotein Analytical Laboratory
Special Studies Section
CMSC 4-125
Johns Hopkins Hospital
600 North Wolfe Street
Baltimore, Maryland 21205

Ship the samples via a commercial overnight delivery service, such as Federal Express. The samples will arrive in the laboratory the following day. Samples should be shipped on Monday or Tuesday, if possible. They should not be shipped on Friday, since there is generally no one in the laboratory to receive them on the weekend. (If it is occasionally necessary to ship on Friday, please contact the Laboratory Director or Laboratory Supervisor in advance so arrangements can be made to receive the samples. The laboratory telephone number is 410-955-3197.)

13.4.1 Certification and Recertification for Sample Shipments

13.4.1.1 Requirements for Certification

1. Read and be familiar with Sections 13.3 & 13.4 of this chapter.
2. Complete training with local Master Trainer.
3. Section C of the Local Laboratory Procedures Checklist (Exhibit 13.1) should be completed by Master Trainer.

13.4.1.2 Requirements for Recertification

1. Prepare one shipment of specimens according to DISC Protocol in previous 3 months.

2. Section C of the Local Laboratory Procedures Checklist (Exhibit 13.1) should be completed by Master Trainer.

13.5 Caution in Handling Blood Samples

It is well known, but nonetheless worth mentioning, that the improper handling of some blood samples, for example those from patients with infectious hepatitis, can lead to infection of personnel who draw, handle or use the samples. Transmission can occur by ingestion, contact or inhalation, and personnel must exercise care when handling blood samples. Never pipet samples by mouth. Avoid contact with plasma. Cover any scratches or cuts on fingers and hands very carefully before handling plasma. Use gloves. Store all samples in sealed containers, and minimize the generation of aerosols by not leaving samples open to the atmosphere longer than necessary.

It has been estimated that it is about 30 times easier to become infected with hepatitis than with AIDS virus through sample mishandling, and it has been recommended that the usual precautions for handling blood specimens to prevent hepatitis infection serve as a guide to prevent AIDS infection as well.

13.6 Supplies

Supplies needed for sample handling and shipment are listed in Table 11.

Table 11

Supplies for Clinical Centers

Disposable Transfer Pipet, Jumbo Bulb Fisher Cat No 13-711-7 5000/case	$189.00/case
Serum Bottle, Wheaton, 2 ml Fisher Cat No 06-406A 288/case	$ 61.69/case
Serum Bottle, Wheaton, 5 ml Fisher Cat No 06-406C 288/case	$ 76.91/case
Rubber Stopper for 2 ml serum bottle Fisher Cat No 06-406-11A 1000/case	$ 36.90/case
for 5 ml serum bottle Fisher Cat No 06-406-11B 1000/case	$ 48.60/case
Aluminum Seal for 2 ml serum bottle Fisher Cat No 06-406-14A 1000/case	$ 24.30/case
for 5 ml serum bottle Fisher Cat No 06-406-14B 1000/case	$ 25.20/case
Cap Crimper for 2 ml serum bottle Fisher Cat No 06-406-21	$135.00
for 5 ml serum bottle Fisher Cat No 10-319-490	$135.00
Plastic Bag (for shipping samples) 8" x 5", zipper lock Fisher Cat No 01-816D 500/case	$ 64.00/case

Table 11 (Continued)

Plastic Bag (for arranging samples for shipment) 2 1/4" x 5" American Scientific Products Cat NoB-1205-1 500/case	$ 29.81/case
Sample Shipping Container Biomailer, polyfoam VWR Cat No 15713-530	Approx $10.00 each
Blood Drawing Tube, 15 ml, red top Fisher Cat No 02-685C 1000/case	$226.40/case
Blood Drawing Tube, 10 ml, red top Fisher Cat No 02-685A 1000/case	$206.60/case
Blood Drawing Tube, 7 ml royal blue (dark blue) top (trace-metal free, no anticoagulant) Fisher Cat No 02-685-17 1000/case	$602.70/case
Blood Drawing Tube, 2 ml purple top Fisher Cat No 02-683-86 1000/case	$192.00/case
Microtainer Serum Separator Tube with Separator Gel (made by Becton Dickinson) 20 tubes/pkg B-D No 5960 Fisher Cat No 02-668-85 200/case	$109.70/case
Fixed Volume Repeating Micropipet, 0.1 ml American Scientific Products Cat No P5064-100	$ 68.00 each
Fixed Volume Repeating Micropipet, 1.0 ml American Scientific Products Cat No P5064-1L	$ 68.00 each
Pipet Tips for Repeating Pipets for 0.1 ml pipet: Cat No P5064-902 1000/pkg	$ 39.00/pkg
for 1.0 ml pipet: tip, Cat No P5064-903P, 750/pkg	$ 36.00/pkg

EXHIBIT 13.1

Dietary Intervention Study in Children

Local Laboratory Procedures Checklist Form

This form is required for laboratory technician certification and recertification. It is to be completed by the trainer observing the individual who is to be certified or recertified perform the actual procedures.

The trainer should be located in a position which allows careful observation of these procedures. No comments should be made by the trainer while these procedures are being carried out.

Identifying Information

1. Clinic Location
   (circle one)

   Johns Hopkins ..... ( )
   Northwestern ..... ( )
   Iowa ..... ( )
   Newark ..... ( )
   New Orleans ..... ( )
   Portland ..... ( )

2. Individual to be certified or recertified

   Name _____
   (please print)

   DISC Staff ID ___ - ___ ___ ___

3. Trainer

   Name _____
   (please print)

   DISC Staff ID ___ - ___ ___ ___

4. Areas for certification (check as many as required).

   a. Blood drawing ..... ( )
   b. Serum preparation ..... ( )
   c. Sample shipment ..... ( )

5. Areas for recertification (check as many as required).

   a. Blood drawing ..... ( )
   b. Serum preparation ..... ( )
   c. Sample shipment ..... ( )

EXHIBIT 13.1 (Continued)

Dietary Intervention Study in Children

Local Laboratory Procedures Checklist

DIRECTIONS: Fill in appropriate section(s) for trainee and submit to the Coordinating Center when all appropriate sections have been completed.

A. Blood Drawing Checklist for the DISC Study

1. Did the phlebotomist note the length of time the subject actually fasted?

   Yes _____ No _____

2. Was the patient appropriately positioned for blood drawing?

   Yes _____ No _____

3. Were the required number of blood tubes obtained?

   Yes _____ No _____

4. Were the blood tubes filled completely?

   Yes _____ No _____

5. Were the blood tubes correctly identified?

   Yes _____ No _____

6. Were the sample I.D. labels fixed to all of the tubes and the sample log sheet?

   Yes _____ No _____

7. Was the serum tube allowed to clot at room temperature for 45 minutes?

   Yes _____ No _____

Comments:

Date: _____ Name: _____

EXHIBIT 13.1 (Continued)

B. Serum Preparation and Storage Checklist for the DISC Study

1. Were the clotted samples centrifuged at the proper speed?

   Yes _____ No _____

2. Did the technician: "Rim" the clot before removing the serum?

   Yes _____ No _____

3. Were the appropriate aliquots prepared for non-lipid tests?

   Yes _____ No _____

4. Was the serum transferred from royal blue top tube to polypropylene vial for Zn+2/Cu+2 analysis?

   Yes _____ No _____

5. Was the hemolysate correctly prepared for RBC folate using the ascorbic acid preservative?

   Yes _____ No _____

6. Were the appropriate aliquots of serum prepared for lipid and apolipoprotein analyses?

   Yes _____ No _____

7. Were the serum bottles/vials correctly labeled?

   Yes _____ No _____

8. Were the glass serum bottles capped with the rubber stoppers and aluminum seals?

   Yes _____ No _____

9. Were the samples stored immediately at -20°C

   Yes _____ No _____

Comments:

Date: _____ Name: _____

EXHIBIT 13.1 (Continued)

C. Serum Shipment Checklist for the DISC Study

1. Were all the vials from a single subject placed into a small plastic bag?

   Yes _____ No _____

2. Was the log sheet properly prepared with

Center name	Yes _____	No _____
Person who prepared shipment	Yes _____	No _____
Date of shipment	Yes _____	No _____
Sample ID	Yes _____	No _____
Visit number	Yes _____	No _____
Name Code	Yes _____	No _____

3. Were all of the small bags containing the subjects' samples placed into a large plastic bag containing the log sheet listing those samples?

   Yes _____ No _____

4. Were the samples packed into the styrofoam shipping container with sufficient dry ice to keep frozen for 48 hours?

   Yes _____ No _____

5. Was the lipid lab notified (by telephone or CC:Mail) of when the samples were shipped?

   Yes _____ No _____

Comments:

Date: _____ Name: _____

DISC DNA filter paper protocol (adapted from CARET study protocol)

Processing EDTA blood for DNA samples

It is of the utmost importance to be sure no sample cards come in contact with any other sample cards! Because of the power of the polymerase chain reaction, any slight contamination may result in erroneous results after amplification of contaminating DNA.

1) Do not centrifuge the EDTA (lavendar top) tube.

2) Process the tube within three hours of collection. If separation of plasma and red cells has begun to occur, invert the tube several times to resuspend.

3) Prepare a clean space on a counter top for spotting the blood card. Assemble a drying rack and attach it to a wall where it will be out of the way. The same rack can be used for multiple samples, and on different days, but EACH SLOT ON THE RACK MAY ONLY BE USED ONCE.

4) Label the top line of the blood card with the subject's name.

5) Behind a shield or face mask, and while wearing gloves, carefully remove the lavendar cap from the EDTA tube.

6) Using a disposable pipet, carefully spot the circles on the blood spot card. To do this, lightly touch the tip of the pipet to the center of each circle and slowly release blood from the pipet until the circle is filled. Do not overfill.

7) Place the spotted card on the drying rack to dry for at least three hours at room temperature. Place the filter card so the spots are facing towards the inside of the rack and the ID label is to the outside. Fold the flap of the card, at the crease at which it folds over the filter paper, all the way over to the back of the card so it, too, is inserted into the drying rack. This minimizes the chances of contaminating an adjacent card. Do not touch the blood spots or let them come in contact with each other. Do not use the same slot on the drying rack more than once.

8) Process the remaining blood as per previous DISC protocols. Restopper the empty EDTA tube and discard it with the transfer pipets in a biohazard container.

9) Clean the counter top.

10) After the blood spot is completely dry, wearing gloves, remove the card from the rack, fold the protective paper back so as to cover the filter paper, and place it in an Ultrabarrier pouch with one Ultrapak dessicant.

11) Peel off the adhesive tape strip. Remove excess air from the pouch by running your hand from the bottom of the pouch to the top, then seal the pouch.

12) With the pouch positioned horizontally and the seal to the back, left-hand side, place a label on the front, top, right-hand corner of the pouch. Place the pouch in a manila envelope for shipment to Baltimore.

13) Place the manila envelope in the -20° freezer with plasma samples. Samples may be stored in a refrigerator for up to 12 hours if immediate freezing is not possible. Do not thaw the samples after freezing.

14) Record the number of dried blood spots to be shipped, and a list of names, to accompany the samples being shipped. Ship blood spot cards frozen in a styrofoam container. Ship early in the week so samples are not left in a mailroom over a weekend.

15) Ship to:

Chris Friedrich, M.D., Ph.D.
Lipid Research Unit, Dept. of Pediatrics
Johns Hopkins Hospital, CMSC 6-104
600 North Wolfe Street
Baltimore, MD 21205

Phone: (410) 614-2521 Fax: (410) 955-1276
e-mail: cfriedri@welchlink.welch.jhu.edu

Please call or send e-mail to notify us when samples are being sent.

DISC Form 60
Rev. 0 02/15/95
1 Page

DISC Blood Spot Sample Shipment Log and Report Form (DNA Ancillary Study)

Center Name: ______________________ Page No.: ________ File No.: ______________

Person who prepared shipment: ______________________

	Month	Day	Year
Date of shipment:	__ __	- __ __	- __ __
Date of receipt:	__ __	- __ __	- __ __

Date of Analysis	Month	Day	Year	Technician No.	Run No.
ApoA-I prom:	__ __	- __ __	- __ __	________	________
ApoE:	__ __	- __ __	- __ __	________	________
ApoA-IV:	__ __	- __ __	- __ __	________	________

Participant ID and Name Code	Visit No.	Sample* Condition	ApoA-I Promoter Genotype (Circle One)	ApoE Genotype (Circle One)		ApoA-IV Genotype (Circle One)	Comments
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	
(Affix Label)		____	G/G G/A A/A	2-2 2-3 2-4	3-3 3-4 4-4	1-1 2-1 2-2	

*CONDITION CODES: S = Satisfactory O = Open T = Thawed W = Wet U = Unsatisfactory for other Reason

KEEP A COPY OF THE SHIPMENT LOG FOR YOUR FILES

13.7 Sample Shipment From Outside the Continental U.S.

13.7.1 Occasionally, a DISC participant may be located outside of the continental US at the time of a scheduled clinic visit. Under these circumstances, the following procedure should be followed.

1. Make arrangements with a physician, clinic or laboratory located near the participant, to draw blood and prepare serum.
2. Have the serum shipped by an overnight transport service such as Federal Express. The sample should be shipped at 2-4° using "blue ice" packs. Use a sufficient number of "blue ice" packs to maintain temperature for 72 hours. Before arranging for the shipment, determine whether the number of packs you will use is sufficient. Do this by setting up a shipping container of the size you will use, packed with a vial of water containing a thermometer. Pack the container with "blue ice", then seal it and allow it to stand at room temperature. Monitor the temperature daily for several days. The temperature should remain constant until the "blue ice" is completely thawed, then it should rise rapidly.
3. Have the serum divided into several separate vials. Use a sufficient number of vials to ensure that you will have enough serum to prepare aliquots for lipids, apolipoproteins and permanent storage. This is a precaution in case one of the vials leaks. Use the type of screw capped polypropylene serum shipment vial that is equipped with a rubber gasket to prevent leakage (see below). Using the usual crimp top sample shipment bottles will probably not be feasible because the local facility would also need a cap crimper.
4. Have the sample sent to your clinic.

5. Upon arrival, note how long the shipment took, and whether the samples arrived cold. Immediately prepare the required aliquots according to usual DISC procedures and store them at -20°C or -70°C until they are sent to the DISC Central Lipid Laboratory.
6. Send the samples to the DISC Lipid laboratory with the next scheduled shipment or within a week, whichever is shorter.

13.7.2 Supplies Required by the Blood Drawing Facility

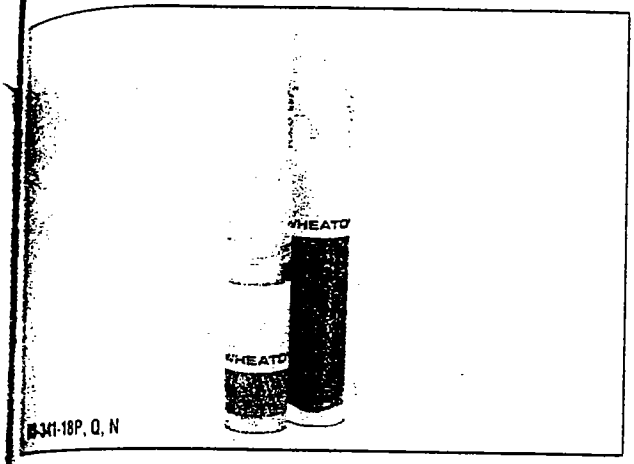
Send the blood drawing facility the following:

1. Vacutainer tubes, needle holder, needles (see Manual Section 13.6).
2. Subject ID labels (see Manual Chapter 4 by visit information).
3. Sample shipment vials, screw capped with rubber "O" ring gasket equipped caps. These are available from various sources, e.g., Fisher Scientific; Fisher cat no. 03-341-18Q (Wheaton no 985747), 4 ml cryogenic vial, free standing base, with polypropylene screw stopper and silicon ring (See Attachment A.)
4. Biological specimen containment materials.

   Biological specimen containment materials include a primary leakproof receptacle, a secondary leakproof receptacle, and absorbent material. See Attachments B (Federal Express instructions for biological specimen shipments), and C (Qorpak price list) for one source of such packaging material.

   Polyfoam shipping container with outside corrugated cardboard packaging.
5. Complete instructions about how your clinic prepares the patient (fasting, posture, etc.), draws blood and prepares serum. Include directions to; separate the serum into the several screw capped sample shipment vials; how to identify the samples; how to use the biological

containment materials; when to ship; and a copy of the attached Federal Express instructions (Attachment B.)



03-341-18P, Q, N

Wheaton Cryule* Internally Threaded Cryogenic Vials

- *Internal threads for greater stability*
- *Streamlined design saves space*

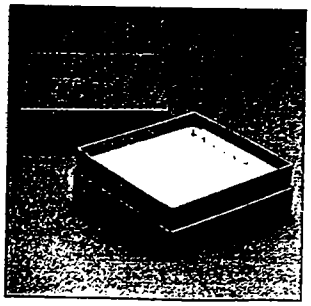
Sterile, streamlined polypropylene vials with polypropylene screw stoppers and silicone rings seal securely. Ideal for preservation of biological specimens with liquid nitrogen at temperatures to −196°C (−320.8°F). Autoclavable at 121°C (249.8°F) for 20 minutes, but stoppers must be loosely loosened or removed to allow pressure equalization upon cooling to prevent wall collapse. White marking area allows sample identification. Available in standard round-bottom style or with flat base that sets vial stand without support on benches. Supplied with caps inserted, in ten bags of 50 per pack.

Capacity	Diameter x H	Wheaton No.	Cat. No.	Pack of 500
Standard Base				
	½ x 1⅞" (12 x 48mm)	985742	03-341-18K	113.76
	½ x 2¾" (12 x 70)	985743	03-341-18L	151.67
	½ x 3⅝" (12 x 90)	985744	03-341-18M	164.32
Freestanding Base				
	½ x 1⅝" (12 x 42mm)	985745	03-341-18N	126.38
	½ x 1⅞" (12 x 48)	985746	03-341-18P	139.05
4 mL Size	½ x 2¾" (12 x 71)	985747	03-341-18Q	151.67

Note: Always use safety equipment, such as gloves, faceshields, and safety cabinets or hoods, when removing vials from storage in cryogenic gases.

Wheaton Cryule Disposable Freezer Boxes

Store externally threaded Cryule round-bottom cryogenic vials with screw caps (03-341-18, -18A, -18B, and -18C on previous page). Square cardboard boxes withstand liquid nitrogen temperatures to −196°C (−320.8°F). Use in freezers or at room temperature. More economical than metal; eliminate cleanup and provide easy handling of vials during shipping.

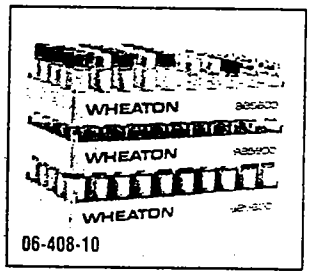


Fits Vial	L x W x H	Wheaton No.	Cat. No.	Pack of 15
03-341-18B	5" x 5⅛" x 1⅞" (13 x 13 x 4.8cm)	651490	12-009-30	51.71
03-341-18C, 03-341-18D	5" x 5⅛" x 1⅞" (13 x 13 x 4.8)	651492	12-009-31	63.53

Prices subject to change. *Call your Fisher Customer Service Center for the latest information.*

Wheaton Cryule Polypropylene Racks

Made of durable polypropylene, each rack has an alphanumeric index that provides easy sample identification and molded corners for stability during stacking. Each is autoclavable at 121°C (249.8°F) for 20 minutes and measures 4L x 7¾W x ⅞"H (10.2 x 19.4 x 2.2cm). See chart below for tube compatibilities.

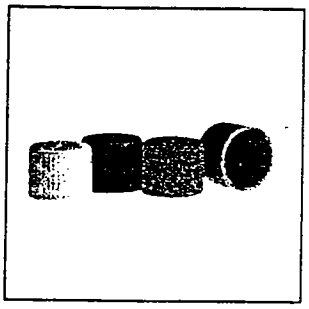


06-408-10

Holds 50	Wheaton No.	Cat. No.	Pack of 5
03-341-18, -18A, B, and C round-bottom vials with screw caps (p. 736), 06-406AA (p. 1948) and 03-337-21 (p. 1958) 12mm diameter autosampler vials, or 12 x 75mm glass culture tubes.	985800	06-408-10	49.00
03-341-18D, E, F, G, H, and J freestanding vials with screw caps (p. 736).	985810	03-341-18S	57.00

Wheaton Cryule Polypropylene Caps

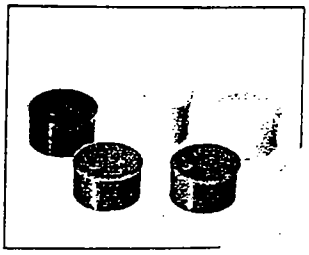
Sterile color-coded 10mm diameter caps for easy identification of individual samples fit externally threaded Cryule round-bottom polypropylene vials with screw caps (03-341-18, -18A, B, and C on previous page). Packed in polybags of 100 pieces per pack. Assorted case contains five bags with 20 each of red, blue, green, yellow, and pink caps.



Color	Wheaton No.	Cat. No.	Pack of 100
Red	242562	06-450-189	10.89
Blue	242564	06-450-190	10.89
Green	242566	06-450-191	10.89
Yellow	242568	06-450-192	10.89
Pink	242572	06-450-193	10.89
Assorted	242576	06-450-194	10.89

Wheaton Cryule Polypropylene Cap Inserts

Use these colored inserts for sample and lot identification of Cryule internally threaded polypropylene vials with screw stoppers and silicone ring seals (03-341-18K, L, M, N, P, and Q at left). Packed in bags of 500 pieces per pack. Assorted pack has 5 bags with 100 each of red, blue, green, yellow, and white inserts.



Color	Wheaton No.	Cat. No.
Red	242582	06-450-195
Blue	242584	06-450-196
Green	242586	06-450-197
Yellow	242588	06-450-198
White	242590	06-450-19[illegible]
Assorted	242592	06-450-[illegible]

As the leader in the overnight Air Express industry, Federal Express has set the following packaging standards to ensure the safe transport of all blood, urine and any diagnostic specimens containing fluids. (Federal regulations may also govern these shipments.)

Recently, there has been a significant increase in such shipments.

These include, but are not limited to, human or animal materials such as excreta, secreta, blood and its components, tissue and tissue fluids being shipped for purpose of diagnosis.

Federal Express requires that the shippers of all blood, urine and other liquid diagnostic specimens package such items to include the following essentials:

1. A watertight primary receptacle.
2. A watertight secondary packaging.
3. An absorbent material must be placed between the primary receptacle and the secondary packaging. If multiple primary receptacles are placed in a secondary packaging, they must be wrapped individually to ensure that contact between them is prevented. The absorbent material, such as cotton wool, must be sufficient to absorb the entire contents of all primary receptacles. It is the responsibility of the shipper to ensure adequate absorbent material is used.
4. A sturdy outside packaging constructed of corrugated fiberboard, wood, metal or rigid plastic ***must*** be used. Styrofoam, plastic bags and paper envelopes are UNACCEPTABLE outer packagings. The minimum acceptable size is 7 x 4 x 2.

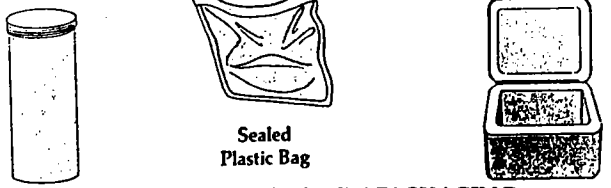
FEDERAL EXPRESS WILL REFUSE TO ACCEPT PACKAGES NOT MEETING THESE REQUIREMENTS.

These illustrations depict samples of packaging which are acceptable for shipping blood, urine or any diagnostic specimens containing fluids. The four requirements outlined below must be used for shipping all such materials via Federal Express. Please note that all of the illustrations shown are acceptable packaging and may be used in any combination as long as the packaging standards are met.

1.

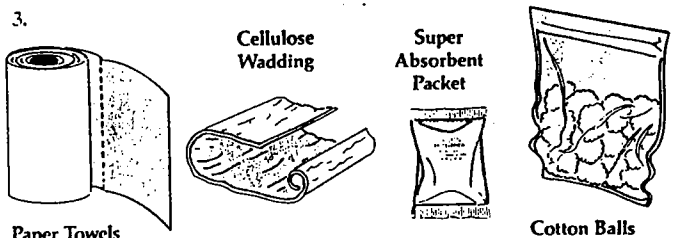
WATERTIGHT PRIMARY RECEPTACLE

2. Slip Cover Can



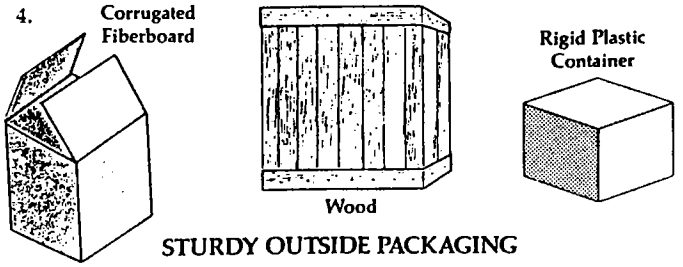
WATERTIGHT SECONDARY PACKAGING

3.



ABSORBENT MATERIAL

4. Corrugated Fiberboard



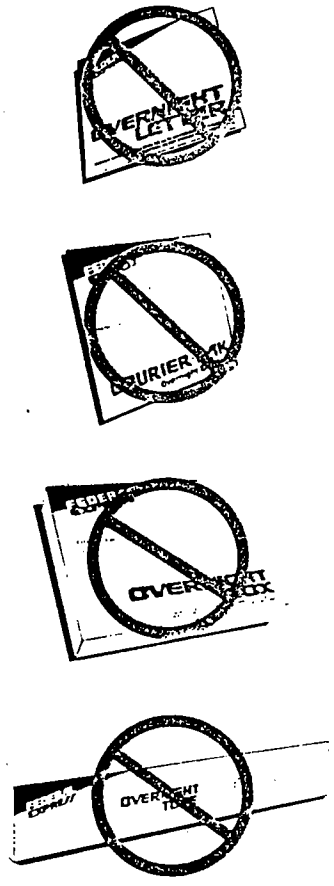
STURDY OUTSIDE PACKAGING

IMPORTANT NOTE

All etiologic agents must be shipped according to applicable Federal regulations regarding infectious substances.

FEDERAL EXPRESS PACKAGING

Federal Express **DOES NOT** provide packaging for blood, urine and diagnostic specimen shipments, and ***will not accept*** these items when shipped in Federal Express supplied packaging such as: Overnight Letters, Courier Pak Overnight Envelopes, Overnight Tubes and Boxes:



SturdeeSeal®

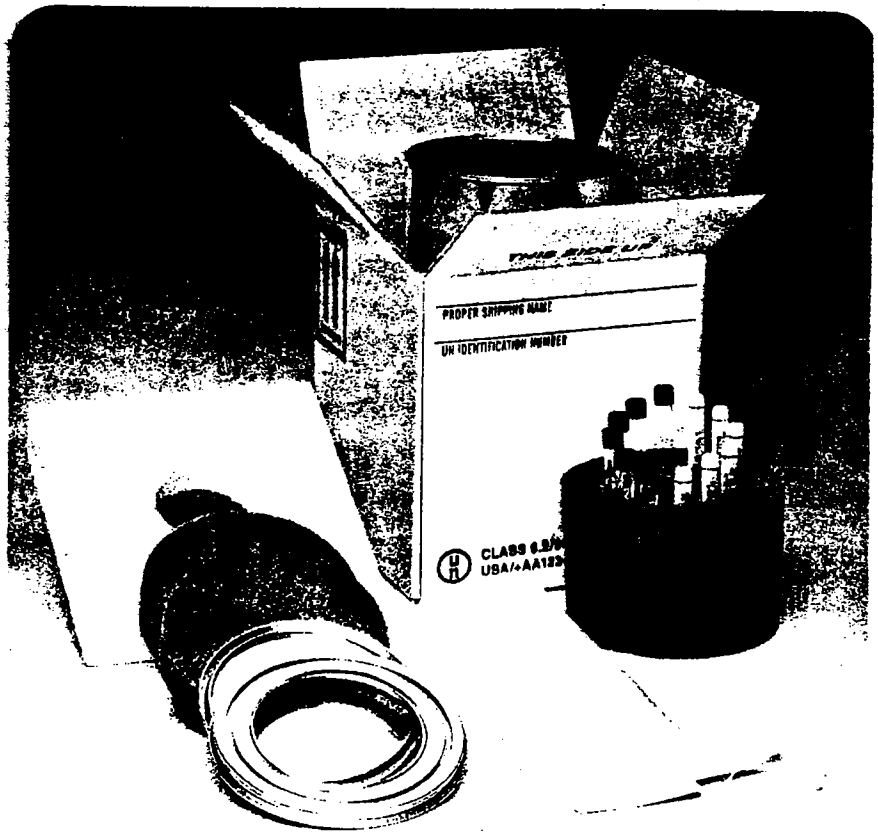
INFECTIOUS SUBSTANCE PACKAGE

CLASS 6.2 MATERIAL

Package CERTIFIED and MARKED

This package comes complete with all components (except vials) to ship your vials and tubes in accordance with Packing Instruction 602 of ICAO, International Civil Aviation Organization and 49CFR Department of Transportation Regulations.

TO ORDER:
ask for Item #6901



(Closing Tool Available for Can and Ring.)

WARNING

All packages are supplied complete (NO VIALS) and the shipper must not make any substitutions. Failure to heed this warning would invalidate package compliance.

The seller makes no claim as to the suitability of this package for the customer's requirements. Determination of the suitability is the responsibility of the Customer.

This package complies with Regulations for Transportation. Other government agencies may have additional warning requirements.

Qorpak

Corporate One West
1195 Washington Pike
Bridgeville, PA 15017-2854

1-800-922-7558

ATTACHMENT C (Continued)

Qorpak®

CORPORATE ONE WEST
1195 WASHINGTON PIKE
BRIDGEVILLE, PA 15017-2854

PHONE: 800-922-7558
FAX: 412-257-3001

INFECTIOUS SUBSTANCE PACKAGE #6901

1995 PRICING

QUANTITY	PRICE EACH	PRICE CASE (12)
12 Each	$ 19.10	$ 229.20
24 Each	$ 17.40	$ 208.80
36 Each (1/2 Pallet)	$ 15.50	$ 186.00
144 Each (2 Pallets)	$ 14.35	$ 172.20

*** Quantities of 12, 24, & 36 will be packed in 12 pack master cartons.**
Minimum order quantity is 12 each or 1 case.