

Attachment B

WHOLE BLOOD COLLECTION AND PROCESSING FOR SERUM DIOXIN

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TUBE METHOD

NOTE: UNIVERSAL PRECAUTIONS SHOULD BE OBSERVED AT ALL TIMES WHEN COLLECTING AND HANDLING BODILY FLUIDS AS OUTLINED BY CDC. REFER TO MMWR VOLUME 36/NO. 2S.

1. COLLECTION PROCEDURE

1. Materials needed per participant.

- Gauze sponges
- Alcohol wipe
- Adhesive bandage
- Red top Vacutainer tubes (eight 10 mL tubes)
- 19g or 21g 3/4" butterfly assembly with multiple sample luer adapter
- Preprinted labels
- Tourniquet
- Vacutainer holder

2. Venipuncture procedure.

- Locate a suitable table and chair for blood collecting and lay out blood collection supplies.
- Locate the puncture site. Hold with 2 fingers on one side of the "alcohol wipe" so that only the other side touches the puncture site. Wipe the area in a circular motion beginning with a narrow radius and moving outward so as not to cross over the area already cleaned. Repeat with a second alcohol wipe.
- Locate vein and cleanse in manner previously described, then apply the tourniquet. If it is necessary to feel the vein again, do so; but after you feel it, cleanse with alcohol prep again, and dry with a sterile gauze square.
- Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry into the skin and pull the skin taut.
- Approach the vein in the same direction the vein is running, holding the needle so that a 15° angle with the participant's arm.
- With bevel facing up, push the needle (either butterfly or regular needle) firmly and deliberately into the vein. Tape the needle in place to allow both hands to be free for collecting the tubes of blood. Activate the vacuum collection tube. If the needle is in the vein, blood will flow freely into the tube. If no blood enters the tube, probe for the vein until entry is indicated by blood flowing into the tube.
- For collection, loosen the tourniquet immediately after blood flow is established and release entirely as the last tube fills. Collect the red top tubes and label with an appropriate label. Place the tubes upright in a rack and allow to clot.

- After the last tube has filled, withdraw the needle with a swift backward motion. When the needle is out of the arm, press gauze firmly on the puncture. Heavy pressure as the needle is being withdrawn should be avoided because it may cause the sharp point of the needle to cut the vein.
- Have the participant raise his/her arm (not bend it) and continue to hold the gauze in place for several minutes. This will help prevent hematomas and bruising.
- Report any reaction experienced by the participant during the venipuncture procedure.
- Label all tubes with the preprinted labels provided, and use a ballpoint pen to add the date collected and your initials to the label.
- Place an adhesive bandage on the participant's arm.

B. SERUM PROCESSING PROCEDURE

1. Materials and Equipment Needed per Participant

- Disposable plastic pipet
- 30 mL (1 oz.) glass vial for **SERUM DIOXIN** with Teflon-lined screw cap
- 2 mL cryovial for **SERUM LIPIDS**
- Preprinted labels
- Centrifuge
- Freezer (-20°C) or dry ice

2. Processing

- After the blood has been allowed to clot at room temperature **for a minimum of 2 hours**, centrifuge the red-top tubes for 15 minutes at the rpm necessary to attain a force of 1000 g. To calculate the number of rpm necessary to attain 1000 g, use the following formula:

$$\text{rpm} = 9450 / r,$$

where r is the distance in centimeters from the center of rotation to the bottom of a test tube when it is extended in the centrifuge head.

Example: for r = 16, rpm = approximately 2400.

- Use a permanent marker to add the date collected and your initials to the labels on all containers.
- To maximize the amount of serum recovered from all of the red top tubes, do the following:
 1. Using a disposable pipet, transfer all of the serum that is free of red cells from each red top tube a labeled 30 mL glass bottle.

2. Any remaining serum left in the red top tubes that has become mixed with red cells should be transferred and combined into a clean 10 mL red top tube. Extra tubes are provided for this purpose.
3. Centrifuge for 10 minutes and transfer the clear serum to the labeled glass bottle containing the serum originally harvested. After all of the serum has been combined into the labeled glass bottle, transfer 1 mL of serum to a labeled 2 mL cryovial for the **Serum Lipids**. Recap each container and freeze upright. Place upright in a -20°C (or lower) freezer and store at the same temperature until shipment to the CDC on dry ice.
4. A collection log is provided. Place a label in the space provided indicating which aliquots were collected for each participant ID. Record in the comments section any variations from the protocol for collection, processing or storage. Once collection is complete, include the collection log with the shipment. Please retain a copy for your records.

SHIPPING INSTRUCTIONS

1. Place the glass bottles in the storage boxes that are provided. Place the 2 mL cryovials in the storage box provided. Place each box inside a zip-lock bag along with an absorbent pad and seal each bag.
2. Place the bagged specimen boxes inside the styrofoam shipping container. Add 10-15 lbs of dry ice to the shipper and place extra packing material around the specimens (newspaper, paper towels, etc.)
3. Prepare a Federal Express airbill for shipping and mark the appropriate boxes including the one for dry ice shipment and overnight delivery. Place a dry ice sticker on the outside of the shipping box.

Shipping address:

Charles Dodson TEL: (770) 488-4305/FAX: (770) 488-4005/EMAIL: wcd1@cdc.gov.
Centers for Disease Control and Prevention
Building 103 Loading Dock
4770 Buford Highway NE
Atlanta GA 30341

4. Please phone, fax or email to the above numbers to inform the day the package is shipped. Ship only on Monday - Thursday to insure that the package will arrive during a regular work day and not over a weekend day or on a Federal Holiday.

Pilot Exposure Investigation: Tittabawassee River
CDC NO. #####-####

SHIPMENT DATE: _____

RECEIPT DATE: _____

SHIPPED BY: _____

RECEIVED BY: _____

S1=SERUM DIOXIN/FURANS/PCBS
=SPECIMEN COLLECTED

S2=SERUM LIPIDS
=SPECIMEN NOT COLLECTED

LABEL	S1		COMMENTS: _____ _____ _____ _____	LABEL	S1		COMMENTS: _____ _____ _____ _____
	S2				S2		
LABEL	S1		COMMENTS: _____ _____ _____ _____	LABEL	S1		COMMENTS: _____ _____ _____ _____
	S2				S2		
LABEL	S1		COMMENTS: _____ _____ _____ _____	LABEL	S1		COMMENTS: _____ _____ _____ _____
	S2				S2		
LABEL	S1		COMMENTS: _____ _____ _____ _____	LABEL	S1		COMMENTS: _____ _____ _____ _____
	S2				S2		
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	S2				S2		
LABEL	S1		COMMENTS: _____ _____ _____ _____	LABEL	S1		COMMENTS: _____ _____ _____ _____
	S2				S2		