

Appendix 6

Blood Collection and Analysis

University of Michigan Dioxin Exposure Study Blood Collection and Analysis

1. Blood Sampling

- 1.1. Blood sampling will take place after the questionnaire has been administered, but before the soil and house dust samples are obtained. Only those respondents that are medically eligible to donate blood will be scheduled for blood sampling. Eighty milliliters of whole blood will be collected, processed for serum and sent out for analysis to the contracted analytical lab. Serum will be analyzed for the 29 dioxin-like congeners recognized by the WHO and the serum percent lipids.

2. Definitions

- 2.1. Phlebotomist: for the purposes of this study, a certified paramedic or nurse contracted to provide phlebotomy services
- 2.2. Phlebotomy team leader: the UMDES staff member who is the primary contact for phlebotomy contractors and respondents
- 2.3. Phlebotomy cover sheet: an information form for collecting and transferring respondent contact information; includes a unique respondent identification number, respondent contact information, time of the scheduled appointment, name of phlebotomist assigned to appointment, space for recording appointment outcomes
- 2.4. Phlebotomy call center: a contracted relay center for scheduling phlebotomy appointments and contacting phlebotomists; capable of receiving phone calls seven days a week, 24 hours per day
- 2.5. Medical eligibility: as defined by the American Red Cross, to be eligible to donate blood the respondent must
 - Weigh at least 110 pounds
 - Had no chemotherapy in the last 6 months
 - Have no history of bleeding or clotting disorders
 - Not currently be taking blood thinner medications
 - Not currently be pregnant or nursing
 - Not currently diagnosed or treated for anemia
 - Have not donated blood within the last 8 weeks

3. Sample Collection and Transport Procedure

- 3.1. Scheduling of the blood collection will take place immediately after consent has been obtained and the questionnaire has been administered
 - 3.1.1. Study personnel will contact the *phlebotomy call center* to schedule an appointment time convenient to the respondent
 - 3.1.2. The call center will be given information necessary to fill out the *phlebotomy cover sheet* including the desired date/time of the appointment
 - 3.1.3. To reduce respondent attrition, most phlebotomy appointments will be scheduled to take place within 48 hours of consent acquisition
- 3.2. At the scheduled appointment time a phlebotomist will be dispatched with the cover sheet to the appropriate meeting place to handle the blood draw
- 3.3. Blood collection will follow a standard phlebotomy protocol observing universal precautions

- 3.3.1. The phlebotomist will first confirm the identity of the study respondent by asking the respondent to verify their name, date of birth and signing of the consent form
- 3.3.2. The phlebotomist will label eight glass, 10 milliliter non-siliconized red-top Vacutainer tubes with their initials, the date and respondent's identification number
- 3.3.3. The respondent will be asked to sit in a suitable chair and designate which arm they would prefer for the venipuncture procedure
- 3.3.4. An accessible vein will be located and the puncture site will be cleansed two times with an alcohol wipe; a tourniquet will be applied to the arm
- 3.3.5. The vein will be punctured using a 21 gauge, multiple sample needle with direct luer adapter
- 3.3.6. Blood collection will continue until the eight, labeled red-top tubes have been filled
- 3.3.7. As each tube is filled with blood it will be placed into a zippered specimen transport bag and allowed to clot for 30-60 minutes.
- 3.3.8. Once eight tubes have been filled the needle will be withdrawn and the puncture site will be covered with sterile gauze and a band-aid
- 3.3.9. Used needles and tube holders will be discarded into a biohazardous waste container for later disposal
- 3.3.10. The respondent will be reminded to keep pressure on the puncture site for several minutes to prevent bruising
- 3.3.11. Any adverse reaction will be recorded on the *phlebotomy cover sheet* by the phlebotomist for follow-up by the *team leader*
- 3.3.12. The cover sheet will then be placed into the specimen transport bag and the package will be transported to the medical laboratory for processing
 - 3.3.12.1. Samples that can not be processed within 60 minutes will be placed on wet ice in a cooler to prevent hemolysis
- 3.3.13. When the sample is dropped off at the medical laboratory the phlebotomist will be required to sign a chain of custody form indicating date, time and name of person relinquished to for processing

- 5.3. Each shipment will be packed with the saved chain of custody forms (white and yellow copies only) sealed in a zip-top storage bag
- 5.4. The cooler will be sealed with packing tape
- 5.5. Coolers will be sent by Federal Express to Alta Analytical Laboratories for high resolution mass spectrometry analysis

6. Data Analysis

6.1. Selected polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) will be measured in serum using high-resolution mass spectrometry (HRMS). Alta Analytical Laboratory of El Dorado Hills, CA will perform the analyses using internal modifications of US EPA methods 8290 (US EPA, 1994) and 1668 (US EPA, 1999) for sample extraction and quantitation. Approximately 25 milliliters of the collected serum sample will be used for the analysis. Samples will be spiked with $^{13}\text{C}_{12}$ -labeled internal standards and the analytes of interest will be extracted with hexane and concentrated. The extracts are then fractionated using silica gel and activated alumina columns. An aliquot of each extract is injected into a gas chromatograph and the selected analytes quantified by HRMS on a Waters Ultima Magnetic Sector High Resolution Mass Spectrometer using selected ion monitoring (SIM) at 10,000 resolving power. The concentration of each analyte will then be calculated based on a standard linear calibration specific to each congener. Each analytical run is blinded to the analyst and consists of the unknown serum samples, a method blank (Quality Control), an ongoing precision and recovery sample (Quality Control), two solvent blanks and two calibration standard solutions. After all quality assurance and quality control (QA/QC) data are reviewed, the analytical results will be calculated on both a whole-weight and lipid-adjusted basis. Serum total lipids for each sample will be calculated using 'Phillips formula' summing triglycerides and total cholesterol. Sample specific estimated detection limits will be reported for each analyte. Any remaining serum will be retained by Alta Analytical for the sole purpose of reanalysis for the specified congeners, if necessary, and discarded within one year of receipt.

7. Quality Assurance/Quality Control Procedures

7.1. Internal analyses

7.1.1. With each analytical sequence, Alta Analytical laboratories will run quality control samples to check the validity of the data output

7.1.1.1. Method Blanks, *an aliquot of matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with the samples. The method blank is used to determine if any analytes or interferences are present in the laboratory environment, the reagents or the apparatus.*

7.1.1.2. Ongoing Precision and Recovery (OPR), *a laboratory blank spiked with known quantities of analytes. Its purpose is to assure that the results produced by the laboratory remain within the limits specified for precision and recovery.*

7.1.2. Congener concentrations will be calculated only after quality control results have been verified

7.2. External analyses

- 7.2.1. An external entity, the National Center for Environmental Health/Center for Disease Control and Prevention laboratories (NCEH/CDC), will be coordinating with Alta Analytical laboratory for quality control purposes
- 7.2.2. NCEH/CDC will send laboratory serum standards to Alta Analytical to verify method accuracy
- 7.2.3. Alta Analytical will return analytical results to the NCEH/CDC labs for comparison
- 7.2.4. Analysis of study serum samples will proceed after Alta Analytical has successfully matched their analytical results to those of the NCEH/CDC laboratories

References

Alta Analytical Laboratory. Analytical Procedures: Modified Method 8290 (Confidential).

Alta Analytical Laboratory. Analytical Procedures: Modified Method 1668 (Confidential).

United States Environmental Protection Agency (US EPA). Method 8290: Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Washington, DC: Office of Solid Waste and Emergency Response, 1994.

United States Environmental Protection Agency (US EPA). Method 1668, Revision A: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS. Washington, DC: Office of Water, 1999.