Standard Operating Procedure for Sample Collection of Heavy Metals (As, Hg, U, Cd, Mn, Se), Creatinine, Phthalate Metabolites, 2,4-DCP, 2,5-DCP and Pyrethroids for Biomonitoring

A. Scope and Purpose

The Four Corner State Biomonitoring Consortium seeks to generate science-based information that will lead to relevant public health policy to address Healthy People 2020 Environmental Health Objectives to ensure safe drinking water, reduce pesticide exposure, and reduce exposure to metals including arsenic, mercury, uranium, cadmium, manganese, and selenium.

Biomonitoring will allow for appropriate data collection and evaluation of environmental chemical exposure burden. The consortium will utilize urine specimen and water sampling techniques to evaluate exposure to environmental chemicals in targeted, potentially higher risk, populations in the Four Corner States region. Trained staff will collect, aliquot, and transport urine specimens and water samples according to an established standard operating procedure outlined below. This procedure provides detailed sample preparation instruction for five environmental exposures of concern: heavy metals (arsenic, mercury, cadmium, uranium, manganese, selenium); creatinine; phthalate metabolites; 2,4-DCP and 2,5-DCP; and pyrethroids.

B. Specimen Collection and Handling of Urine Sample Best Practices

- **First Morning Specimen:** For best results, this is the specimen of choice for urinalysis. The first morning void is the most concentrated; it contains higher levels of analytes. The participant will be instructed to empty their bladder immediately prior to going to sleep. If the participant has an atypical work/sleep schedule, he/she will be instructed to utilize the 8-hour specimen technique. Over an 8-hour period, the participant will collect all urine that is voided. Any voided urine should be pooled, refrigerated and logged, so that a true 8-hour specimen is recorded.

C. Preparation

Laboratory (LAB) prepares sample kits prior to Epidemiologist Investigator (EPI) arrival.

1. Assemble kit with included items list (Appendix A).
2. Print sample number (customer code) with coding system: “BMSSXXXYYY”.
    i. SS = State
    ii. XXX = FIPS County code
    iii. YYY = Unique to participant
3. Print water label number with coding system: “WSSXXXYYY”.
    i. SS = State
    ii. XXX = FIPS County Code
    iii. YYY = Unique to participant
4. Place labels on the urine specimen containers, falcon tubes and water sample containers specific to the participant ID for each sample collection.
5. Add label to water sample container, “NEED PRESERVATIVE ADDED IN LAB”.

6. Epidemiologist Investigator(s) (EPI) receives sample kit for field collection from laboratory (LAB).
7. EPI inspects sample collection kit(s) and supplies list (Appendix A) for inclusion of all materials, completing Sample Collection Kit Supplies Checklist
    i. See Appendix for Sample Collection Kit supplies checklist.
8. Place EPI labels on participant information sheet and laboratory intake form.
9. Sort pre-labeled containers in field collection boxes.
10. For transport cooler:
    i. Lay dry ice to cover the bottom of the cooler.
    ii. Place terrycloth towel over dry ice.

D. **Water Sample Collection in the Field for Heavy Metals (As, Hg, U, Cd, Mn, Se)**

1. Identify primary drinking source in household and avoid sources of contamination of sample source prior to collection.
2. Collect sample from faucet which is high enough to put the collection bottle underneath without contacting the mouth of the container with the faucet.
3. Turn the tap on a steady stream of **COLD** water and run for at least 2-3 minutes.
4. Wear disposable, powder-free nitrile gloves.
5. Fill collection bottle with water sample to the shoulder line of the bottle.
6. Proceed to section E to collect for arsenic speciation.

E. **Environmental Water Sample Collection in the Field for Arsenic Speciation**

1. Pour water sample into XXmL beaker to black line. Place cap securely back on water sample container.
2. Using a disposable plastic 30mL syringe, extract water from beaker.
3. Push 30mL of water through MetalSoft Center arsenic speciation cartridge into 250mL clean, plastic bottle.
4. Seal bottle with tape and place water samples securely in transport cooler.
5. Rinse plastic beaker with deionized water then discard DI water.
6. Remove gloves and dispose of properly.

F. Urine Specimen Collection in the Field for Heavy Metals (As, Hg, U, Cd, Mn, Se), Creatinine, Phthalate metabolites, 2,4-DCP, 2,5-DCP, and Pyrethroids

Instructions for Field Technician:

Initial Visit

1. Inform participant of biomonitoring, sampling procedure, and test results protocol (See Appendix).
2. Obtain patient consent (See Appendix).
3. Administer exposure assessment survey (See Appendix).
4. Review Urine Sample Collection Instruction sheet with participant (See Appendix).
5. Schedule sample pickup time with participant and schedule a reminder notification.

   i. Reminder notification should be administered 12 to 24 hours prior to scheduled pick-up time. Participant should be notified by chosen method of contact on test results protocol.
6. Inform patient of first morning specimen for urine collection protocol.
   i. This is under ideal conditions. In consideration of timing, cost and participation rates, a spot specimen collection is acceptable. This will be noted in the Urine Sample Information sheet (Appendix).
7. Demonstrate proper uncapping, capping and sealing of the container in biohazard Ziploc bag, using an example container.
8. Review Urine Collection Checklist with participant (See Appendix A).
9. Match label ID on specimen container to participant ID on forms prior to handing container, form and bag to participant.
10. Complete First Visit section of Sample Collection Process Checklist (Appendix).

Specimen Retrieval

1. Upon return, verify that forms were properly completed and sample procedure was followed appropriately.
2. Wear powder-free nitrile gloves for specimen handling.
3. Match participant ID on container with participant ID on completed forms and specimen container.
4. Pack refrigerated sample in transport cooler on dry ice.
5. Remove gloves and dispose of properly in biohazard waste bag.
6. Complete Urine Sample Information sheet (Appendix).
7. Complete Sample Collection Process checklist (Appendix).

G. Aliquoting in the Field for Mercury in Urine

1. Lay out adsorbent table pad on flat surface.
2. Wear powder-free nitrile gloves for specimen handling.
3. Remove urine specimen from biohazard bag and match participant IDs on falcon tube and urine container.
4. Place falcon tube containing sulfamic acid in rack, removing screw cap.
5. Using 10mL syringe, aliquot 4.5mL of urine from specimen container into falcon tube.
6. Cap falcon tube and apply sealing tape to cap and container.
7. Remove gloves and dispose of gloves and syringe in biohazard waste bag.

PROTOCOL FOR SAMPLE ALIQUOTING AND SHIPPING IN LABORATORY

Aliquoting Procedure for Urine Samples for Heavy Metals (As, U, Cd, Mn, Se), Mercury, Creatinine, Phthalate Metabolites, 2,4-DCP, 2,5-DCP, and Pyrethroids

Completed by Laboratory Technician or Chemist in laboratory.

1. Complete Sample Aliquoting Worksheet.
2. Wear safety glasses, disposable lab coat, and disposable, powder-free nitrile gloves. Change gloves if contamination occurs.
3. Prepare clean work space, lay out absorbent pad and place all necessary collection samples on workspace (pipettes, specimen collection vessel, (7) 15mL metals-free falcon tubes).
4. A total of 5 aliquots are needed, separately for urine and water. Prepare as listed below: *Will change based on our decision/restrictions for specific tests.

URINE

Test	Procedure
Metals	No Aliquot
Mercury	Add urine to 4.5mL line on falcon tube which already includes preservative. Securely cap the tube and then mix well by inversion to dissolve preservative mixture.
Creatinine	No Aliquot
Phthalate Metabolites	With disposable transfer pipet, transfer 4mL urine into 15mL falcon tube.
2,4-DCP	With disposable transfer pipet, transfer 4mL urine into 15mL falcon tube.
2,5-DCP	With disposable transfer pipet, transfer 4mL urine into 15mL falcon tube.
Pyrethroids	With disposable transfer pipet, transfer 4mL urine into 15mL falcon tube.

WATER

Test	Procedure
Metals	No Aliquot
Mercury	Add 10mL of water to falcon tube.
Arsenic	Aliquoting done in field.

SAMPLE PACKAGING AND TRANSPORTATION PROTOCOL

Laboratory will follow CDC LRN-C Specimen Shipping and Transport procedures and ship aliquots to appropriate testing site, as listed below.

Test	**Testing Site**	**Shipping Frequency**
Urine – Metals	AZ, CO, NM, UT	----------
Urine - Mercury	AZ, CO, NM, UT	----------
Urine - Creatinine	AZ, CO, NM, UT	----------
Urine - Phthalate Metabolites	AZ	
Urine - 2,4-DCP	CO	
Urine -2,5-DCP	CO	
Urine - Pyrethroids	NM, UT	
Urine – Arsenic		
Water - Metals	AZ, CO, NM, UT	----------
Water – Mercury	AZ, CO, NM, UT	----------
Water – Arsenic Speciation	AZ, CO, UT	----------

Four Corners States Biomonitoring Consortium

SAMPLE ALIQUOTING WORKSHEET

Participant ID: _____

Test	Scheduled	Completed
Urine Metals		
Urine Mercury		
Urine Creatinine		
Urine Phthalate Metabolites		
2,4-DCP		
2,5-DCP		
Pyrethroids		
Water Metals		
Water Mercury		