Improving the Collection and Management of Human Samples Used for Measuring Environmental Chemicals and Nutrition Indicators

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Contents

Executive Summary

The Centers for Disease Control and Prevention (CDC)'s *Improving the Collection and Management of Human Samples Used for Measuring Environmental Chemicals and Nutrition Indicators* describes important factors for obtaining and using high-quality samples in studies that assess environmental exposures and nutrition status. This document does not provide all-inclusive guidance for designing and conducting studies. Recognizing important considerations for collecting, storing, managing, and transporting human samples can minimize external pre-analytical contamination risks, ensure analyte integrity, and promote accurate exposure and nutrition assessments. This document is intended for use by epidemiologists, laboratorians, and other health scientists in state or local public health programs that are involved in the design and implementation of human biomonitoring studies. Further, users should be familiar with the concepts described in the Association of Public Health Laboratories (APHL) *Guidance for Laboratory Biomonitoring Programs* (https://www.aphl.org/aboutAPHL/publications/Documents/EH_2012_Guidance-for-Laboratory-Biomonitoring-Programs.pdf).

General and test-specific considerations are included in this document for key components of sample handling including required materials, sample collection and processing, storage, and transport. These factors highlight the importance of seeking laboratory input at the earliest stage of design of a study or investigation.

Introduction

Biomonitoring is widely recognized as an important tool for assessing people's nutrition status and exposure to natural or man-made chemicals in the environment. By measuring substances or their biomarkers in human specimens, such as urine, blood, or serum, public health officials and scientists may be able to identify nutrition deficiencies and unusual or potentially harmful exposures that may cause disease or increase disease risk.

When conducting biomonitoring studies, use of specific and sensitive analytical methods is critical to ensure accurate exposure or nutrition assessment. For accurate performance of the methods, biological specimens must be collected and managed in a manner that preserves their quality and minimizes external contamination. Study staff must consider all factors, before, during, and after collection, that may affect the quality and integrity of the samples and target analytes. A single specimen may be collected and then used for measuring multiple analytes, and some analytes may be more vulnerable to contamination during collection or analysis than others. Pre-analytical planning can anticipate contamination and analyte stability issues and take measures to avoid jeopardizing the quality of analytical measurements. Additionally, proper processing, storage, and transport is required to maintain sample integrity. The purpose of this document is to provide individuals involved in the design and conduct of biomonitoring studies with considerations for collection and management of biologic samples to ensure that they are of the highest possible quality for analytical measurement.

This document is not intended to be an all-inclusive guide or protocol for designing and executing high-quality human exposure studies; rather, it is meant to describe considerations for protecting the quality of clinical samples throughout collection, storage, and shipping. In addition, these considerations

highlight the importance of involving laboratory personnel in the earliest stage of design of a study or investigation.

Planning for Collection of Human Samples

Protocols for Collection and Handling of Samples

When designing a biomonitoring study, investigators select chemical analytes and biological matrices based on the chemical or biomarker of interest, feasibility of collecting specimens, and alignment with the purpose and goals of the study. As described in APHL's *Guidance for Laboratory Biomonitoring Programs, c*omprehensive project planning for a biomonitoring study also includes development of pre-analytical protocols.

Sample collection and processing protocols provide detailed instructions regarding how, where, and when samples are to be collected and processed. Input from laboratory staff is essential to identify requirements that may vary for each biomarker (i.e., contamination risks, required materials). Protocol for collection may include specification of needed materials, volume required for each matrix, labeling requirements, etc.

Sample storage and handling protocols specify the conditions and length of storage time for preserving the integrity of the samples.

Sample transport protocols describe requirements for transporting samples to the laboratory for further analysis. Protocols may specify timing requirements, necessary packaging materials, and documentation needs.

Multi-Analyte Testing

Frequently, a single sample is used for multiple analytical tests. Study protocols should specify analytical priority in case the volume of specimen collected is not sufficient for all desired testing or if there are not enough aliquots available for all desired tests. In addition, protocols should consider contamination concerns and storage needs that may vary from test to test.

In biomonitoring studies where multiple tubes of blood are collected, it is important to collect the specimen needed for measuring priority analytes in the first or second tube in case problems (e.g. vein collapse) arise during collection. This is especially important when collecting from individuals who may not be able to provide the desired amount of sample (e.g., children or older adults).

In biomonitoring studies that require multiple aliquots of urine, the aliquoting protocol should specify that the priority analyte aliquots are dispensed first in case limited urine is collected. This is especially important when collecting from children or older adults.

Use of Stored Samples

If the study utilizes previously collected and archived samples, protocols should consider timing of sample collection, current condition of samples, potential contamination issues, and the presence or absence of preservatives. Review records of historical storage conditions, including any freeze-thaw cycles that may affect analyte or matrix integrity.

Collection Material Lot Screening and Testing

Screening, or lot testing, of collection materials is important in biomonitoring studies because many target analytes (i.e., trace elements, other chemicals) are ubiquitous in the environment. Using untested containers or collection devices to collect, store, or analyze samples could introduce contamination and result in erroneous analytical values. Lot screening allows the user to pre-screen collection materials for contamination prior to their contact with samples. Lot screening is performed by measuring the concentration of the target analyte(s) in a screening solvent before and after contact with the device or container being tested. Contamination in the device is observed as a higher concentration in the solvent that has been in contact with the device. The screening solvent is typically water or another reagent that mimics solubility characteristics of collected samples. Metal contamination in most commercial collection devices is usually from materials used to make the collection device or anticoagulants contained in them. Conditions of the manufacturing processes for commercial devices and containers can differ with each batch produced. Therefore, contamination that enters the devices and containers during manufacturing can differ also. Consequently, screening these devices and containers each time a new lot of materials is manufactured is necessary. CDC screens, or lot tests, 40-50 units from each "lot" of the following for possible trace metals contamination:

- Syringes/Butterflies/Needles for blood collection
- Alcohol wipes (used to disinfect the site prior to venipuncture)
- Urine collection devices
- Blood tubes/micro-containers
- Processing supplies
- Analysis consumables (e.g., analytical reagents)
- Storage vials

Safety

Planning for an investigation or study should address measures to protect the safety of laboratory and field staff who handle biological specimens. Measures may include required use of personal protective equipment (PPE) and completion of training in safety topics such as blood-borne pathogens and hazardous waste disposal. Additional information about universal safety precautions for handling biological specimens is available from the Occupational Safety and Health Administration (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051).

Sample Collection: Reducing Risk of Contamination and Maintaining Sample Integrity

General Contamination Concerns

Sampling technique, environment, and materials may introduce contaminants that lead to erroneous results. Potential sources of contamination include:

- Environments conducive to cross-matrix contamination (e.g., births, surgeries, and IVF treatments)
- Medical devices, IVs, catheters, or other tools

- Various chemicals used in the manufacture of plastic materials (e.g., di-2-ethylhexyl phthalate [DEHP], bisphenol A [BPA]) that leach from collection materials
- Antimicrobials or preservatives in supplies, such as soap used for hand-washing, wipes, and toothpaste
- Inadequate handwashing by technician collecting the sample

Other Potential Problems during Collection

Errors during sample collection of all types may render analytical testing impossible or incorrect. Errors may include:

- Collecting insufficient quantity of sample for desired test(s)
- Failure to label a sample correctly and to provide all pertinent information required on the test request form
- Use of incorrect container/tube for appropriate sample preservation
- Failure to tighten container lids, resulting in leakage and/or contamination of samples
- Failure to maintain the sample at the appropriate temperature requirement

General Considerations for Urine Collection

At a minimum, record the manufacturer, model number, lot numbers, and expiration dates of collection devices. Collect urine according to instructions provided for the individual test/analysis.

Preservatives

In order to avoid possible degradation, urine preservatives may allow for maintenance of samples at room temperature, providing comparable analytical results to those obtained with fresh or refrigerated samples. Commonly used preservatives for chemical urinalysis samples include tartaric acid, boric acid, chlorhexidine, ethyl paraben, thymol, and sodium propionate. Preservation times are typically within the range of 24 to 72 hours. Claims for the duration of stability for specific analytes should be obtained from the manufacturer. If a preservative is required, it is important that the designated preservative be in the urine collection container at the start of the collection. The preservative may be toxic and caustic; do not spill or discard the preservative. In urinalysis testing, inversions of the capped container ensure that the preservative is properly mixed with the urine. Typically 8-10 gentle inversions is sufficient. Some preservatives' protocols may call for vigorous shaking as opposed to gentle inversions.

Note that preservatives are not universally used for all urine samples. Further, the presence of a preservative may preclude analysis for some chemicals. For example, the use of an ethyl paraben preservative could render samples ineligible for quantification of select chemicals (e.g., parabens, BPA).

For samples that require freezing shortly after collection and prior to transport, freeze the urine according to the protocol procedures, after collection. Pack vial(s) appropriately to ensure the urine remains frozen during transport to the laboratory.

Sources of Error and Contamination

Contamination risks vary according to the sampling plan for each particular study; however, common sources of error and/or contamination during collection of urine samples include:

- Failure to collect sample as specified in the study protocol (e.g., a clean-catch midstream sample or complete 24-hour collection/aliquot)
- Inadequate refrigeration of unpreserved sample
- Failure to add preservative to urine collection container prior to collection of the sample
- Inappropriate use of preservative
- Insufficient mixing of specimen with preservative
- Insufficient quantity of sample to meet minimum fill line on preservative transport container
- Use of inappropriate collection container
- Failure to refrigerate sample when bacteriological examination of the specimen is required
- Failure to tighten sample container lids, resulting in leakage
- Failure to provide subjects with adequate instructions for 24-hour urine collection
- Failure to divide sample into separate containers for tests with different storage requirements
- Failure to divide sample into homogeneous aliquots; it is recommended to gently mix the urine cup prior to each split

General Considerations for Blood Collection

Record the manufacturer, model number, lot numbers, and expiration dates of collection devices. Collect whole blood according to instructions provided for the individual test/analysis. To prevent clotting, mix the blood sample to ensure good distribution of the additives throughout the blood; typically, the recommendation is to invert the tube 8-10 times after drawing the blood sample. Label each vial after collection, wrapping the label around the vacutainer in an upright position (so that the barcode appears like a vertical ladder) so that electronic scanners can read the barcodes. Place blood tubes in storage boxes and maintain the samples at the temperature specified by test protocol. Avoid freezing blood tubes because it may cause tubes to crack. If cool packs are frozen, wrap them in paper or other materials so that they will not directly touch the blood tubes, or allow enough time for them to warm to refrigerator temperature before placing them near whole blood tubes. To avoid risk of potential hemolysis, do not place whole blood samples in direct contact with cool packs.

Order of Blood Draw

It is critical that the blood collection be followed as specified in the study protocol. Each study protocol may have required blood draw specifics based on the needs of the study. Order of draw is a vital component to phlebotomy that mitigates the possibility of cross contamination, which causes erroneous and unreliable test results.

For studies requesting blood or serum for metals testing, follow the order of draw specified in the study collection protocols to avoid possible contamination from collection devices that are not pre-screened for metals.

In designing a study collection protocol, one can start with the general guidance from the Clinical and Laboratory Standards Institute (CLSI) guidelines (excerpted below) and adapt as needed to meet specific study needs. According to the CLSI, the order of draw should be as follows for both glass and plastic tubes:

Tube Priority	Type	Description
1	Blood Culture bottles	Blood Culture: bottles (green & orange caps) containing 40 mL of liquid broth to be used as a set, or individually (green cap) for aerobic-only cultures.
2	Isolator tubes	Isolater tube: 10 mL (adult) or 1.5 mL (pediatric) glass tube with yellow and black stopper containing liquid. Used for mycobacteria, fungus, or AFB blood cultures.
3	Royal blue top (no additive)	Royal blue top tube with No Additive: used for trace metal serum determinations.
4	Blue top (3.2% sodium citrate)	Blue top tube with 3.2% sodium citrate: used for coagulation testing and other plasma or whole blood determinations. If only a coagulation specimen is being drawn, draw 1-2 mL into another blue top (3.2% sodium citrate) tube, discard, and then collect the specimen.
5	Red top (no additive) and Gold top (SST)	Serum tubes: include Red top tubes (no additive) and SST (gold top) gel separator tubes with clot activator. Used for serum determinations including chemistry testing, serology, and therapeutic drug monitoring.
6	Light Green and Green top (sodium heparin)	Green top tube with sodium heparin: used for plasma or whole blood determinations.
7	Lavender top (EDTA), Tan top (EDTA), Pink top (EDTA) and Black top ESR-Vacuum tube	EDTA tubes: includes Lavender top, Pink top (used for blood bank testing), Tan top (used for lead testing), and Royal Blue top with EDTA (used for trace metal whole blood or plasma determinations). Black top ESR-Vacuum top with 3.2% sodium citrate: used for Westergren sedimentation rate.
8	Gray top (sodium fluoride)	Gray top tube with potassium oxalate/sodium fluoride: used for lactic acid testing and other plasma or whole blood determinations.
9	Yellow top (ACD) Solution A	Yellow top tube with ACD (acid citrate dextrose) Solution A: used for whole blood determinations including flow cytometry and tissue typing assays.
10	Yellow top (ACD) Solution B	Yellow top tube with ACD (acid citrate dextrose) Solution B: used for whole blood determinations including flow cytometry assays.

Plasma and Whole Blood Preparation

For plasma and whole blood, draw an adequate amount of blood in the appropriate evacuated tube to yield the necessary volume. Next, gently mix the blood collection tube by inverting immediately after collection, as indicated by the test protocol. Inadequate mixing of tubes with anticoagulants may allow microclots to form and interfere with the analytical method. Most samples require separation of plasma from cells within two hours of collection, while some require separation within 15-30 minutes. Tubes intended for whole blood analyses should not be centrifuged.

Common technician errors that can affect specimen integrity include:

- Under-filling the tube, thereby creating an additive to blood ratio that results in excessive volumetric dilution of the blood (QNS or "quantity not sufficient"), or a concentration of additive that interferes with the analytical test
- Failure to collect sample with the correct additive
- Failure to mix sample with additive immediately after collection
- Failure to separate plasma from cells within timeframe specified in protocol
- Failure to label transport tubes as "plasma"
- Failure to indicate type of anticoagulant

Serum Preparation

For serum collection, draw an adequate amount of blood in the appropriate evacuated tube to yield the necessary serum volume. Invert tube (generally 5-10 times) to activate clotting. Blood should be allowed to clot at room temperature for approximately 30 minutes.

Mix any tube containing additives immediately after collection. Inadequate mixing of tubes with separator gel may interfere with barrier formation. This may cause gel material to remain in the serum or plasma layer.

Common technician errors affecting the quality of serum samples include:

- Failure to separate serum from red cells within the recommended time after venipuncture
- Failure to allow samples to clot before centrifugation
- Failure to centrifuge the samples at the recommended g-force for the recommended time for serum and plasma separation (typically 1000-1300 g-force). It is recommended to check with the centrifuge rotor manual (for RCF to RPM table) for the proper RPM to use with the specific rotor.

Freezing Prepared Serum or Plasma

If the protocol calls for freezing of serum or plasma:

- Consider freezing the sample as soon as it is separated from the cells after placing in a plastic tube (cryo-vial).
- Freeze samples in cryo-vials unless instructed otherwise.
- For multiple analyses, it is suggested to aliquot the sample into multiple cryo-vials prior to freezing, if feasible. Aliquoting can reduce the number of freeze-thaw cycles that the sample is subjected to and facilitates distribution of samples to multiple laboratories for analyses.

Container Type and Mixing

When the closed evacuated collection system is used for collection of a variety of tubes (with different additives) from one subject, it is important to fill the tubes in an order that minimizes any adverse effect on sample quality due to the additive from the preceding tube.

Potential Sources of Error/Rejection

Hemolysis

Hemolysis occurs when the membrane surrounding red blood cells is disrupted, and hemoglobin and other intracellular components escape into the serum or plasma. Hemolyzed serum or plasma varies in color from faint pink to bright red, rather than the normal straw color. Grossly or moderately hemolyzed specimens may be rejected and even slight hemolysis may alter certain test results.

Hyperbilirubinemia

Icteric serum or plasma varies in color from dark to bright yellow, rather than the normal straw color. Icterus may affect certain measurements. Upon receipt of such specimens, the laboratory may request a new sample to assure results of diagnostic value.

Turbidity (Lipemia)

Turbid, cloudy or milky serum (lipemic serum) may result from the presence of fatty substances (lipids) in the blood. A recent meal may produce transient lipemia; therefore, it is recommended, though not always feasible in an epidemiologic study, that subjects fast 12-16 hours before a blood specimen is obtained. Bacterial contamination may also cause cloudy serum. Moderately or grossly lipemic specimens may alter certain test results.

Radioisotope Interference

Diagnostic procedures or therapy involving radioactive compounds may invalidate radioisotope assays. Obtain specimens for anticipated radioisotope assays in advance of such procedures.

Quality Assurance Measures

Quality assurance (QA) includes procedures to promote, monitor, and evaluate the overall quality of the laboratory testing process, including pre-analytic, analytic, and post-analytic phases. QA measures may focus on control of contamination, use of proper materials for collection and storage, and analyte stability. For example, the following tools may be used to evaluate sample processing procedures, examine potential external contamination, and assess the accuracy and precision of the measurement:

- **Field blanks** are containers used to assess potential contamination during sample collection, processing, and storage before analysis. Containers may be filled with solvent (water or another solvent known to be free of the target analyte(s)) in the field and analyzed as regular samples to determine whether any of the processes between collection and analysis can result in external contamination. Alternatively, in specific situations (e.g., when conducting metals testing), unused collection containers of the same model and lot as the other materials are transported back to the laboratory for addition of solvent and subsequent testing. Field blanks are particularly useful when pre-screened collection materials are not available.
- **Sample splits** can be used to assess the quality of aliquoting technique and the precision of the analytical measurement. Samples split in the field undergo the same pre-analytical and analytical treatment. They may be particularly useful to independently assess the reproducibility of the analytical measurement.

- **Blind QCs** are another tool to evaluate the accuracy and precision of the measurement. Blind QCs are materials of the same matrix (e.g., urine, blood) as the human samples that are generally added to the analytical run by the field staff or the laboratory and are subjected to the same analytical processing as the collected human samples. Laboratory analysts are not aware of which samples are the blind QC. Blind QCs may be particularly useful to identify sample mix-ups or errors in the sequence of samples. Blind QCs are also useful to show the consistency of the laboratory analytical processes over time or as an external quality assurance challenge for the participating laboratory.

Post-Collection: Sample Storage and Shipping

General Storage Considerations

Urine

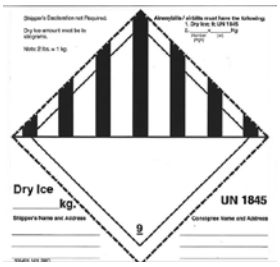
- After urine collection, store all urine specimens in a freezer (preferably at ≤ -20°C) to pre-freeze sample for shipment. Consult the laboratory protocol.
- If samples are collected by study participants at their homes, ensure that participants receive instructions for proper storage and transfer to study staff.
- Externally threaded containers are preferred because they are less prone to contamination of the specimen and to leaks. (Internally threaded containers can develop leaks when biological material dries within the threads, compromising resealing.)

Blood

- Do not freeze blood in blood collection tubes because it could cause them to crack. Transfer blood/blood products to plastic pre-screened cryo-vials before freezing. Store samples in blood tubes (i.e., evacuated tubes) in refrigerator at approximately 4˚C and ship on cold packs. Store blood samples in vials (i.e., cryo-vials) preferably at ≤20˚C and ship frozen (i.e., on cold packs or dry ice).
- Minimize the amount of time whole blood is stored prior to processing it to serum or plasma if needed.

Shipment

Place sample vials inside a storage box in an upright position. Use rubber bands to securely close the box(es). Place the storage boxes inside a biohazard bag with absorbent material. Ship the storage boxes with the sample vials in an insulated shipper. Use DOT compliance labels; when shipping boxes, use the following labels as appropriate:



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- Refrigerate “Do not freeze”: for blood samples in collection tubes, ship with cold packs
- Keep frozen: for urine and serum samples in cryo-vials, ship with dry ice
- Over pack label
- UN3373 Biological substance Cat. B (the label needs to be rotated to a diamond position to read UN3373 horizontally)
- Up-arrow label (only needed if the shipping box does not have the Up-arrows)
- Dry Ice Label (Record weight in kilograms of dry ice alone; include sender/receiver addresses)

Clinical Laboratory Improvement Amendments (CLIA)-compliant laboratories and CDC ship specimens at the conditions specified below:

- Blood in original tubes: with cold packs at approximately 4˚C (new plastic tubes are typically more fragile with freezing)
- Blood in cryo-vials: with dry ice
- Serum in separator tubes: with dry ice
- Serum in cryo-vials: with dry ice
- Urine: with dry ice

Consult your protocol because shipment conditions may vary depending on the specific analytes being measured. To ensure that frozen samples remain frozen during shipping, allow one pound of dry ice for every two hours in transport.

Test-Specific Considerations

Whole Blood: Lead/Cadmium/Total Mercury/Manganese/Selenium/Cobalt/Chromium

Collection

- No fasting or special diets are required before collection of blood.
- Use sterile, lot screened collectors for sample acquisition. Lot screening of materials is highly advised. If lot screening is not possible, use collection devices that are labeled as “metal free,” “for trace elements,” or “for lead testing.” The designation of “sterile” does not indicate that the device is free of metals contamination.
- Include one or more field blanks (empty collection tubes) so potential metal contamination can be evaluated in the analytical phase.
- Avoid heparin anticoagulant; EDTA anticoagulant is preferable.
- If the focus of the study is metals, collect blood tubes for metals analysis first.
- A specimen is deemed unacceptable when there are low sample volumes, suspected contamination due to improper collection procedures or collection devices, and/or contamination during sample preparation/analysis.
- Specimen contact with dust or dirt may compromise test results. It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots).
- Do not analyze clotted samples by this method due to the inhomogeneity issues.

Storage and Shipping

- Do not freeze blood in blood collection tubes because it increases the risk that the tubes could crack. Transfer to plastic, pre-screened cryo-vials before freezing. Store specimens in blood tubes (i.e., evacuated tubes) in refrigerator at approximately 4˚C and ship on cold packs.
- Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL). Avoid colored plastics and containers containing o-rings, when possible, because of the increased risk of trace element contamination from coloring agents or o-ring materials.
- Store blood samples in vials (i.e., cryo-vials) at ≤-20˚C and ship on either cold packs or dry ice.
- Thawing and refreezing samples has not been found to compromise sample results.

Whole Blood: Speciated Mercury (Inorganic Mercury/Methyl Mercury/Ethyl Mercury)

Collection

- Consider contamination from metals found in collection materials. Screening collection materials is highly recommended.
- Use sterile, lot screened collectors for specimen acquisition. Lot screening collection materials is highly recommended. If lot screening is not possible, an alternative is to use collection devices that are labeled as “metal free,” “for trace elements,” or “for lead testing.” The designation of “sterile” does not indicate that the devices are free of metals contamination.
- Avoid heparin anticoagulant; EDTA anticoagulant is preferable.
- If the focus of the study is metals, collect blood tubes for metals analysis first.

Storage and Shipping

- Store samples in refrigerator at 4˚C and ship on cold packs.

Whole Blood: Volatile Organic Compounds (VOCs)

Collection

- Studies of VOCs indicate that their half-life in human blood is extremely short. It is therefore suggested that samples be obtained either before removal from exposure or as quickly after this time as possible.
- Verify that blood collection tubes obtained from commercial sources do not contain VOC contamination that can greatly interfere with the ability to obtain analytical results indicative of the degree of exposure. Blood collection tubes can be specially cleaned to remove potential VOC contaminants.
- Anticoagulant: use a mixture of sodium oxalate and sodium fluoride. This anticoagulant is chiefly intended to stop metabolism so that VOC levels do not change appreciably during storage. This mixture's ability to prevent clotting of blood is not as great as many other anticoagulants, but it has low VOC properties because it is made up of salts. Thus, once samples have been collected, they must be mixed thoroughly to allow the complete dissolution and distribution of the anticoagulant. If a blood mixer/rocker is available, samples should be placed on this mixer/rocker for at least 3 minutes. If a mixer is not available, the blood can be mixed by hand approximately 30 times to completely mix the anticoagulant into the blood sample. Check the bottom of the tube to insure that the anticoagulant powder is completely dissolved. **If it appears that it is not (it may appear as a white spot on the side of the tube), tap the end of the tube gently in the palm of the hand until it breaks loose and dissolves**.
- Isopropanol used to disinfect the venipuncture site has resulted in interferences in the analytical measurement by introduction of this compound into samples. This contamination can be easily prevented by swabbing the site with a dry sterile gauze bandage and allowing the site to dry for 5 - 10 seconds after disinfection with isopropanol.

Storage and Shipping

- Keep samples at refrigerator temperatures during storage and shipment.
- All samples should be placed in a cooler on cold packs or into a refrigerator within 30 minutes of sample collection.
- Samples should not be frozen or stored at freezer temperatures at any time during sample collection and shipment.
- Preliminary experiments have indicated that the concentration of some volatile analytes changes over sample storage time. Therefore, the samples should be shipped within 1-2 days of collection so that they can be analyzed within 8 weeks of collection.

Blood Cell Collection for Acrylamide/Glycidamide/Ethylene oxide/Formaldehyde

Collection

- No fasting or special time of day for specimen collection is required.
- Blood cells from EDTA-whole blood or heparinized whole blood are needed.

Storage and Shipping

- Specimens collected in the field can be shipped refrigerated at 5 ºC or frozen on dry ice.
- Specimens can be kept refrigerated for 3 days.
- For long term storage, samples are stored at -70 ºC. Samples are stable for at least 5 years if stored at -70 ºC.
- Two to three freeze-thaw cycles did not show any changes in values in the in-house experiments. However, multiple freeze-thaw cycles of diluted whole blood samples possibly increase the formation of blood clots, which complicates the analysis of the samples. Therefore, diluted whole blood samples should not undergo more than 3 freeze-thaw cycles.

Whole Blood: Folate

Collection

- EDTA whole blood is used for folate determination. No fasting or special time of day for specimen collection is required.
- Ideally, the whole blood is processed on the day of collection. However, intact whole blood can be kept refrigerated for a few days without compromising folate stability.
- Whole blood folate is measured in a hemolysate prepared with ascorbic acid solution (1% w/v). The ascorbic acid solution should be prepared fresh daily (e.g., add exactly 30 mL of deionized water to 0.3 g of L-ascorbic acid in a 50-mL Falcon Tube and mix to dissolve the ascorbic acid). It should be kept at room temperature during use and any leftover should be discarded at the end of the day.
- To generate the hemolysate, exactly 100 µL of well-mixed EDTA whole blood is added to exactly 1 mL of ascorbic acid solution (1% w/v) in a plastic cryo-vial and the pipette tip is rinsed 3 times with the hemolysate. Mix by vortexing 15-20 seconds.
- A hematocrit result is needed to calculate red blood cell folate from whole blood folate and needs to be obtained for each patient from freshly collected EDTA whole blood. Ideally, serum folate should also be measured so that the amount of folate in serum can be subtracted from the amount of folate in whole blood for an accurate calculation of red blood cell folate.

Storage and Shipping

- The hemolysate vials must be frozen immediately at -20˚C or lower. Folate in the hemolysate is sensitive to degradation at room temperature and especially at elevated temperature.
- For long-term storage (more than a few months), freeze samples at -70°C. Samples are stable for at least 3 years if stored at -70˚C.
- The hemolysate vials must stay frozen until analysis, thus samples must be shipped frozen on dry ice.
- Up to 3 short (<2 hours) freeze-thaw cycles are acceptable if samples need to be reanalyzed.

Serum: Cotinine, Hydroxycotinine

Collection

- To avoid contamination, only persons who do not use tobacco products should collect and handle samples.

- No fasting or special time of day for specimen collection is required.
- If collecting from both users and nonusers of tobacco, note tobacco user status on log sheet.
- Use 10 mL red top (no anticoagulant) Vacutainer® tubes (*one completely filled 10 mL red top tube will yield, on average, 4 mL of serum).*
- Allow blood to clot at room temperature (for a minimum of 2 hours if possible [blood can be centrifuged sooner as long as the blood has completely clotted]).
- Centrifuge the red-top tubes for 15 minutes at the rpm necessary to attain a force of 1000 g.
- To maximize the amount of serum recovered from multiple red top tubes, use a disposable pipet, transfer as much of the liquid serum that is free and clear of red cells from the red top tubes to a new unused red top tube from which the serum splits will be made. (If you notice that there is a jelly-like mass on top of the clot, gently press down on this mass with a disposable pipet to express the trapped serum, and centrifuge the tube again.
- Any remaining serum that has become mixed with red cells should be transferred to a different new 10 mL red top tube.
- Centrifuge the bloody serum for 10 minutes, and transfer the clear serum to the other red top tube containing the serum originally harvested.

Storage and Shipping

- Freeze all samples at ≤-20°C (≤-70°C is better).
- Three freeze-thaw cycles did not show any changes in values in the in-house experiments.
- Ship frozen on dry ice.

Saliva: Cotinine, Hydroxycotinine

Salivette® devices are used for the collection of saliva samples. The Salivette® is made up of a sponge piece that fits in a suspended (removable) plastic insert that sits in the primary tube that will be centrifuged to force the saliva out of the sponge. After the container has been centrifuged, the suspended insert, which contains the sponge piece, should be removed and discarded. You may need a pair of hemostats or forceps to remove the insert. The saliva should have collected in the bottom of the plastic tube.

Collection

- Saliva should not be collected until 30 minutes after eating or drinking anything.
- No fasting or special time of day for specimen collection is required.
- If collecting from both users and nonusers of tobacco, note tobacco user status on log sheet.
- Use a Salivette® to collect saliva (*one Salivette®, when completely saturated, will yield approximately 1.5 to 2.0 mL of saliva).*
- Have the participant remove the sponge from the Salivette® and place in the mouth. The sponge should be chewed or placed under the tongue until it becomes saturated with saliva and saliva starts to pool in the mouth and you can no longer prevent yourself from swallowing. This may take up to 2 minutes.
- Remove the saturated sponge and place into the suspended insert. Place the cap on the tube; label the **OUTSIDE** tubes with the participant ID.

- Centrifuge tubes at 3500 rpm for 15 minutes. It is recommended to check with the centrifuge rotor manual (for RCF to RPM table) for the proper RPM to use with the specific rotor.
- The recovered saliva can be transferred either by pouring directly into a 2 mL cryo-vial or a pipet may be used if desired.

Storage and Shipping

- Freeze all samples at ≤-20°C (≤-70°C is better)
- Three freeze-thaw cycles did not show any changes in values in the in-house experiments.
- The Salivettes® may be frozen at ≤-20°C (≤-70°C is better) without centrifuging. However, transferring saliva to 2 mL cryovials is recommended and will reduce the amount of storage space required for the specimens.
- Ship Salivettes® or vials frozen on dry ice.

Serum: PBDEs/OCP/PCBs, Lipids

Collection

- No fasting or special time of day for specimen collection is required.

Storage and Shipping

- If possible, use 10mL amber glass bottles.
- Keep specimens frozen for storage and shipping (preferably -20˚C for temporary storage and -70-80˚C for long-term storage).

Serum: Vitamin C

Collection

- No fasting or special time of day for specimen collection is required.
- Vitamin C is sensitive to oxidation and only stable in acid-treated serum. Metaphosphoric acid (MPA) is used to stabilize serum. This is a strong acid and needs to be handled with care.
- The MPA solution (6% w/v) can be made ahead of time (3 g MPA in 50 mL deionized water; 30 minutes for the crystals to go into solution) and stored refrigerated for one week. A portion of the MPA solution is removed every day, kept at room temperature during use, and any leftover should be discarded at the end of the day. Because MPA is usually 34-37% pure by weight, the actual MPA fraction of the 6% solution is ~2%.
- Add exactly 100 µL of freshly prepared serum to a plastic cryo-vial. Then add exactly 400 µL of MPA solution to the serum and mix vigorously for 15-20 seconds.

Storage and Shipping

- Serum samples must be frozen immediately at -70˚C or lower. Samples are stable for at least 3 years if stored at -70˚C.
- Serum samples must stay frozen until analysis, thus samples must be shipped frozen on dry ice.
- Up to 3 short (<2 hours) freeze-thaw cycles are acceptable if samples need to be reanalyzed.

Serum: Nutritional biomarkers (ferritin, soluble transferrin receptor, folate, vitamin B6, vitamin B12, methylmalonic acid, vitamin A, vitamin E, carotenoids, vitamin D, fatty acids including trans fatty acids)

Collection

- No fasting or special time of day for specimen collection is required for most nutritional biomarkers; however, serum folate and serum fatty acids cannot be correctly interpreted in a non-fasted person and vitamin E data are more interpretable if expressed relative to total cholesterol.
- Ideally, the whole blood is processed on the day of collection. However, intact whole blood can be kept refrigerated for a few days without compromising the stability of the serum nutritional biomarkers.
- Avoid serum separator collection tubes (SST) for trans fatty acids. If SST is not avoidable, assess for potential interferences from the separator gel.

Storage and Shipping

- Serum samples must be frozen immediately at -20˚C or lower. For long-term storage (more than a few months), freeze samples at -70˚C. Samples are stable for at least 3 years if stored at -70˚C.
- The least stable serum nutritional biomarkers are folate, vitamin B6, most carotenoids, and polyunsaturated fatty acids. For optimum sample stability, samples should be stored at -70˚C, even short-term.
- The most stable serum nutritional biomarkers are ferritin, soluble transferrin receptor, vitamin B12, methylmalonic acid, vitamin A, vitamin E, and vitamin D. If necessary, samples can be stored refrigerated for a few months without compromising sample stability.
- Serum samples must stay frozen until analysis, thus samples must be shipped frozen on dry ice. For methylmalonic acid and vitamin D, serum samples can be shipped at ambient temperature.
- Up to 3 short (<2 hours) freeze-thaw cycles are acceptable if samples need to be reanalyzed.

Serum: Aflatoxin B1-Lysine

Collection

- Every effort should be made to minimize hemolysis during sample collection. Serum samples with extensive hemolysis present may result in analysis interferences and possible invalid results.
- Plasma cannot be used in place of serum. EDTA interferes with the hydrolysis step during sample preparation and will yield an invalid result.

Serum: Per- and Polyfluoroalkyl Substances

Collection

- No fasting or special time of day for specimen collection is required.
- Avoid PTFE/Teflon® and polyvinylidene fluoride (PVDF) coated materials

Storage and Shipping

- Samples should be refrigerated as soon as possible and then transferred to labeled specimen containers, preferably within 4 hours of collection for storage.
- Samples should be stored frozen, preferably in polypropylene or polyethylene containers.
- Glass containers may be used if the specimens are to be analyzed for other environmental chemicals for which storage in plastic may not be an option.
- PTFE/Teflon® and polyvinylidene fluoride (PVDF) coated materials should be avoided.

Serum: Copper, Selenium, and Zinc

Collection

- No fasting or special diets are required before collection of blood.
- Use sterile, lot screened collectors for sample acquisition. If lot screening is not possible, use collection devices that are sold as "metal free" or "for trace elements." The designation of "sterile" does not indicate that the device is free of metals contamination.
- If the focus of the study is metals, collect blood tubes for metals analysis first.
- Draw the blood into a pre-screened 7-mL evacuated tube. A 3-mL evacuated tube size will not produce the optimal volume of serum for this test. Allow the blood in the stoppered evacuated tube to clot for 30-40 minutes, but not longer than 60 minutes. Without opening the evacuated tube, centrifuge it for 10 minutes at 2400 rpm. It is recommended to check with the centrifuge rotor manual (for RCF to RPM table) for the proper RPM to use with the specific rotor. Use a pre-screened serum separator to remove the serum from the clot. Under a laminar flow hood, pour the serum in the serum separator into pre-screened polyethylene vials.

Storage and Shipping

- Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL). Avoid colored plastics and containers containing o-rings, when possible, because of the increased risk of trace element contamination from coloring agents or o-ring materials.
- Transport serum samples at ≤ 4˚C.
- Once received, store at ≤ -20˚C until time for analysis.
- Thawing and refreezing samples has not been found to compromise sample results.

Urine: Antimony, Arsenic (total), Barium, Beryllium, Cadmium, Cesium, Chromium, Cobalt, Lead, Manganese, Molybdenum, Nickel, Platinum, Strontium, Thallium, Tin, Tungsten, and Uranium

Collection

- No fasting or special diets are required before collection of urine.
- Use sterile, lot screened cups if available for sample acquisition. The designation of "sterile" does not indicate that the devices are free of metals contamination.

Storage and Shipping

- Transport urine samples frozen (packed in dry ice during shipment is preferred, when possible).
- Once received, store long term at ≤ -20°C until time for analysis. Short-term storage at 2– 8°C is acceptable (e.g. two weeks). Refreeze at ≤ -20°C portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.
- Lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL cryogenic vial or 15 mL centrifuge tube) are preferred for analytical aliquots if available. Avoid colored plastics and containers containing o-rings, when possible, because of the increased risk of trace element contamination from coloring agents or o-ring materials.

Urine: Speciated Arsenic

Collection

- No fasting or special diets are required before collection of urine unless the focus is on the inorganic arsenic species and their metabolites. In that instance, request specimens from individuals that have not consumed large amounts of seafood within the past 72 hours.
- Use sterile, lot screened collectors for sample acquisition. The designation of “sterile” does not indicate that the devices are free of metals contamination.
- Freeze urine samples immediately upon collection.

Storage and Shipping

- Transport urine samples frozen (packed in dry ice during shipment is preferred, when possible).
- Once received, store long term at ≤ -70°C until time for analysis. Refreeze at ≤ -70°C portions of the sample that remain after analytical aliquots are withdrawn. Avoid excessive thawing and refreezing to prevent intraconversion between arsenic species.
- Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL cryogenic vial or 15 mL centrifuge tube).

Urine: Mercury and iodine

Collection

- No fasting or special diets are required before collection of urine.
- Use sterile, lot screened collectors for sample acquisition if available. The designation of “sterile” does not indicate that the devices are free of metals contamination.
- To prevent loss of mercury from the urine before analysis, add preservative solution to urine as soon as possible or within 10 hours of collection in the proportion of 10 µL of preservative solution per 1 mL of urine (example: To a tube containing 50 µL of preservative, up to 5 mL of urine can be added for urine mercury analysis). The preservative solution is 200 g/L sulfamic acid, 0.01% Triton® X-100. Mix the urine well after addition of the preservative.

Storage and Shipping

- Transport urine samples frozen (packed in dry ice during shipment is preferred, when possible).

- Once received, store long term at ≤ -20°C until time for analysis. Refreeze at ≤ -20°C portions of the sample that remain after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.
- Acceptable containers for analytical aliquots include lot screened polypropylene (PP) cryo-vials or tubes (e.g., 2 to 5 mL cryogenic vial or 15 mL centrifuge tube).

Urine: Caffeine and metabolites, Phytoestrogens, Non-Persistent Pesticides, Organophosphate Flame Retardants, Phthalates, Phenols and Personal Care Products Chemicals, Polycyclic Aromatic Hydrocarbons, 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)

Collection

- Wash hands with water and air dry before handling urine cup (avoid soaps and towels/absorbent materials).
- Avoid using towelettes/wipes to clean genital area before urination.
- Avoid polycarbonate and polyvinyl chloride plastic materials.
- Random collection during the day is acceptable.

Storage and Shipping

- Keep specimens frozen for storage and shipping (preferably -20 °C for temporary storage and -70-80°C for long-term storage).

Samples for Chemical and Radiological Response

Collection

- Following a chemical exposure incident, collect blood and urine samples for each adult involved. For children, only collect urine samples unless the CDC says otherwise. See https://emergency.cdc.gov/chemical/lab.asp for more specific guidance for collecting, packing, and shipping specimens related to a chemical emergency.
- In addition to unique patient identifiers (e.g., medical records number, specimen identification number) specimen labels should convey the collector's initials, date, and time of collection so that law enforcement officials may trace the specimen to the collector should investigations lead to legal action and the collector has to testify that he or she collected the specimen.
- Maintain a list of names with corresponding specimen identification numbers at the collection site so that results can be reported to patients. It is recommended that you record additional data for use in the interpretation of results. Additional data may include: time of potential exposure, method of urine collection if other than "clean-catch", indication if sample was collected post-mortem, and antidotes administered prior to sample collection.

Storage and Shipping

- Store blood samples at 1°C to 10°C. Do not freeze.
- Freeze urine samples optimally at -70°C.

Definitions

Accuracy – The closeness of mean test results to the actual (true) value (concentration) of the analyte

Aliquot – Any part or portion of a sample

Analyte – A substance, such as a chemical, measured by a laboratory method

Analytical sensitivity – The lowest analyte concentration that can be measured with acceptable accuracy and precision

Analytical specificity – Measuring only the *correct* component and includes examining the effects of potentially interfering substances on the measurement

Biomonitoring – The direct assessment of nutrition indicators or exposure to environmental chemicals by measuring the substances or their biomarkers in biological specimens, such as blood or urine

Precision – The closeness of repeat individual measurements of an analyte in multiple aliquots of a single homogeneous volume of biological matrix

Quality assurance – Procedures to promote, monitor, and evaluate the overall quality of the laboratory testing process, including pre-analytic, analytic, and post-analytic phases

Quality control – Procedures to monitor and evaluate the quality of the analytical testing process of each method to assure the accuracy and reliability of test results

Reliability – The degree to which the result of a measurement can be depended on to be accurate

Sample – Any material—biological, clinical or environmental—taken as a representation of a whole, including aliquots and derivatives, used for analysis or medical diagnosis

Specimen – Discrete portion of a body fluid, breath, hair or tissue taken for examination, study or analysis of one or more quantities or properties assumed to apply for the whole

Stability – Measured concentrations of analytes are not altered by conditions that a sample is likely to encounter during analysis: sample collection and handling, short-term room temperature storage, long-term freezer storage at a specified temperature, and after three freeze-thaw cycles