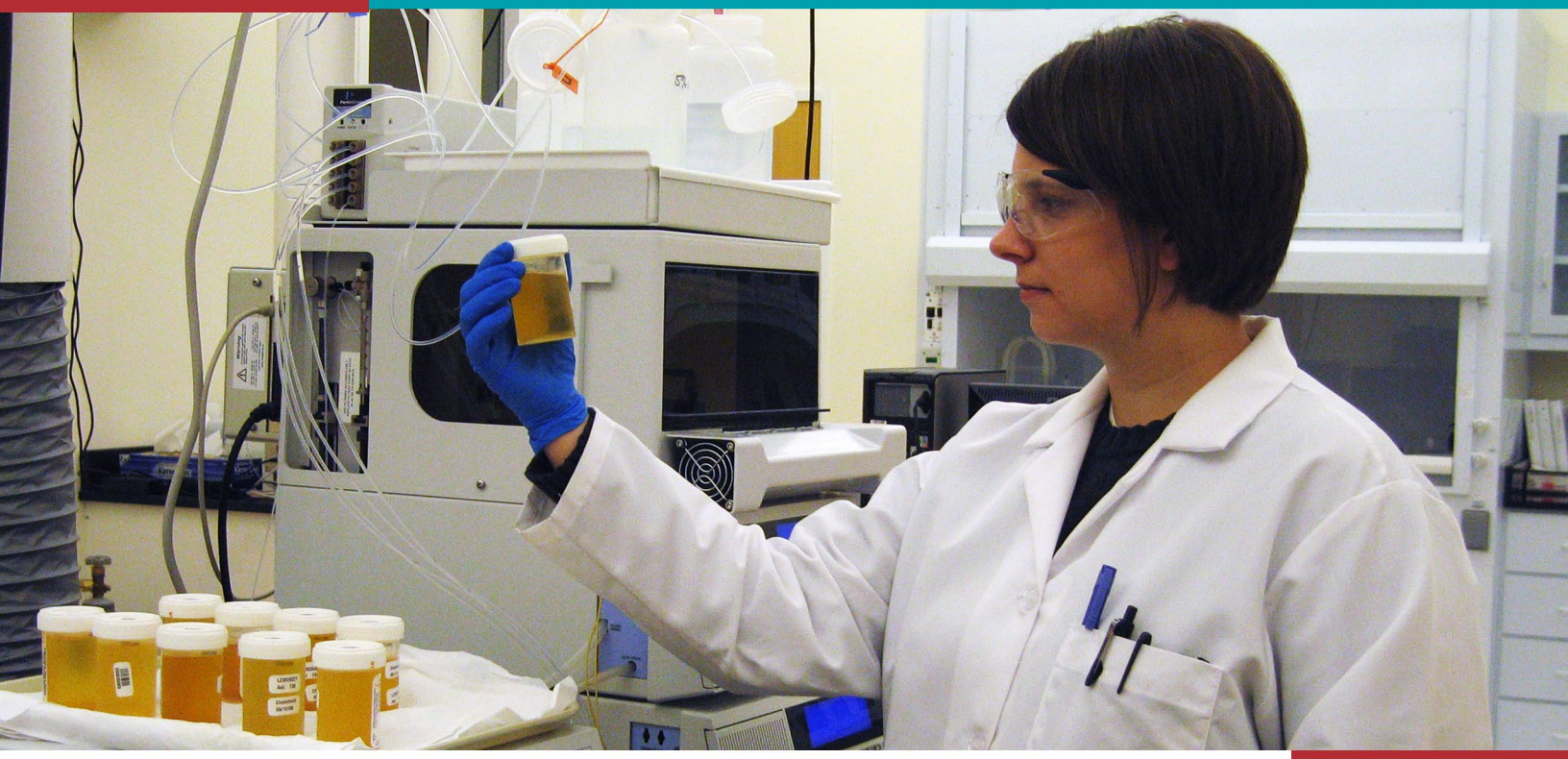
GUIDANCE FOR LABORATORY BIOMONITORING PROGRAMS

APHL Report



APRIL 2012

Developing Biomonitoring Capabilities

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

The Association of Public Health Laboratories (APHL) is a national non-profit organization dedicated to working with members to strengthen governmental laboratories that perform testing of public health significance. By promoting effective programs and public policy, APHL strives to provide member laboratories with the resources and infrastructure needed to protect the health of US residents and to prevent and control disease globally.

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PREAMBLE

Public health professionals, policy makers and the public are increasingly concerned about human exposure to chemicals in our environment. While personal care products, food packaging and other consumer conveniences have improved some aspects of our quality of life, they have also introduced the opportunity for many new and not well understood exposures.

Recent studies, including the Centers for Disease Control and Prevention's (CDC) *National Report on Human Exposure to Environmental Chemicals*[1], confirm that widely used chemicals such as bisphenol A and flame retardants are routinely found in human blood, urine or tissue. These studies are important first efforts in answering critical environmental health questions, but do not provide targeted or regional information, health effects information, or exposure sources. It is essential that state and local public health organizations have the tools necessary to investigate environmental health questions and problems in their respective communities.

Biomonitoring measurement and subsequent trend analysis improve our understanding of the relationship between exposure to environmental chemicals and health. Biomonitoring identifies and quantifies chemicals and elements in the human body to provide scientific evidence of exposure to a particular product or chemical. Conversely, biomonitoring can also offer reassurance that despite the presence of a chemical in the environment, it is not accumulating in the human body.

For more than a decade, the Association of Public Health Laboratories (APHL) has supported biomonitoring; it is the gold standard for assessing human exposure to chemical substances, such as lead and pesticides. The data are useful to establish baselines, for research on health effects, and in risk assessment and management. Information about our levels of exposure helps prioritize studies of chemicals and their potential health effects. These studies also allow decision-makers to create public health and environmental policies based on sound science and to target limited public resources to reduce unusual exposures. The data are also valuable in assessing the efficacy of a public health intervention, as in the removal of lead from gasoline and indoor smoking restrictions. Finally, biomonitoring data can create incentives for industry to explore alternatives to toxic chemicals.

In 2009, APHL developed a National Biomonitoring Five-Year Plan to establish a network of public health laboratories able to provide accurate human exposure data that will inform public health decisions through biomonitoring. This guidance document was developed for laboratories interested in establishing biomonitoring programs in accordance with that Plan.

APHL has worked with national partner organizations to ensure broader support for and understanding of a national biomonitoring system that reflects the integration of the disciplines, technologies, and expertise needed to better utilize biomonitoring in practice and policy. The outcome of a national biomonitoring system will be a more coordinated approach in the design and development of exposure studies, a more effective use of limited resources, higher quality data, and improved practice, data sharing throughout the system, and eventually a healthier population. As such, APHL partnered with other non-profits to convene their members to discuss biomonitoring. Additional reports and publications can be found on the APHL website[2].

APHL will continue its efforts in establishing a national biomonitoring system to improve environmental public health practice and policy. This Guidance document is a critical component to assuring an efficient and effective system among state and national laboratory partners by supporting high-quality, standardized laboratory practice. This Guidance document is intended as a reference for public health laboratories interested in biomonitoring.

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[1]Department of Health and Human Services Centers for Disease Control and Prevention. The Fourth National Report on Human Exposure to Environmental Chemicals, Data Sources and Data Analysis—Limit of Detection. 2009. Available at http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf. Accessed on 9/8/2010.

[2]http://www.aphl.org/aphlprograms/environmental-health

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INTRODUCTION

About the Guidance Document

The intended audience of this guidance is a laboratorian working in any state or local public health or environmental laboratory:

- with or without existing biomonitoring laboratory capability,
- with or without current funding for biomonitoring analysis.

Additionally, this guidance is written so that it can be utilized by any state or local public health or environmental laboratory that may become involved in the design and implementation of biomonitoring studies. The content was developed to be useful for analytical laboratories new to biomonitoring, as well as those with previous experience. It is assumed that users of this guidance have an understanding of good laboratory practices and basic principles of analytical chemistry. A background in epidemiology is not necessary, but basic knowledge and access to epidemiological expertise is beneficial and recommended. The document is formatted in a way as to provide quick reference to specific topics of interest. We expect this to be a valuable tool to which analytical laboratories will refer frequently.

The goals of this guidance are to outline the infrastructure and expertise needed to develop laboratory capacity for a biomonitoring program and to highlight some of the main considerations chemists should address before beginning a biomonitoring study, which include the need to:

- Define the goals of a biomonitoring study;
- Choose the appropriate biomarker in the appropriate matrix at a sufficient level of sensitivity;
- Identify resource needs and sources of potential funding;
- Collaborate with epidemiologists and toxicologists in the development of the study design and to analyze and communicate biomonitoring data;
- Produce reliable, valid and comparable data;
- Engage the community early in the study design process; and
- Develop an effective communication plan that involves reporting of individual results (if appropriate), aggregate data and access to public health, citizens or medical professionals for results distribution and interpretation.

While the technology and instrumentation used for biomonitoring testing have a lot in common with environmental testing laboratories, biomonitoring presents a set of unique challenges. Assaying clinical matrices involves the safe handling of potentially infectious materials, different interferences, and possibly the analysis of metabolites. The greatest differences between biomonitoring and environmental testing involve the interpretation of clinical findings and effective communication of results on an individual and community basis.

Good biomonitoring programs are not exclusively laboratory efforts, but rather a collaboration among many public health disciplines. Working with state and local health officials, academia and community leaders can help to identify potential political, cultural and economic barriers and identify solutions for successful implementation of a biomonitoring program.

The Use and Value of Biomonitoring in Public Health Assessments

Biomonitoring efforts are initiated to answer various environmental health questions. The analytical data generated are useful in disease investigation, assessing exposure in a population and for public health policy. The most common applications of biomonitoring by state and local public health agencies include general surveillance, targeted investigations to characterize exposure in a potentially exposed and at-risk population, and emergency response.

Public Health Surveillance

Biomonitoring may be conducted for the purpose of detecting and measuring spatial or temporal differences in population exposure or as a way to evaluate the efficacy of public health actions to reduce exposure.

For example, the National Health and Nutrition Examination Survey (NHANES) routinely collects data on the health and nutritional status of the U.S. population, including exposure to chemical substances. Data are collected from approximately 5,000 representative individuals through interviews, physical exams, and clinical tests every year in NHANES. Environmental chemical analytes are measured in blood, serum, and urine samples from NHANES participants (per two-year survey period) (http://www.cdc.gov/exposurereport/).

NHANES uses a cross-sectional sample of a defined population: the design most often used in surveillance studies. A cohort design, in which the same individuals are followed over time, may be used at the state or local level but is less common due the greater expense and losses to follow-up that can compromise validity of the results. In some states, mandatory physician and laboratory reporting of biological measures (e.g., blood lead or pesticide poisonings) is a source of biomonitoring surveillance data.

Targeted Public Health Investigation of a Potentially-Exposed Community

Biomonitoring may also be conducted in response to health concerns in a community resulting from the discovery of environmental contamination or a cluster of disease with a possible chemical exposure origin. Emergency response activities may also be initiated in response to a single acute exposure to a toxic substance.

The purpose of the investigation may be to measure the range and distribution of the exposures in the community, to determine whether exposures are elevated above a reference level, or to recommend public health action. Examples of a convenience sample might include responding to a small-scale exposure event as is described in Case Study 1.

Case Study 1

The Massachusetts Department of Public Health (DPH) responded to an incident at a large middle school involving a mercury spill. DPH environmental health staff sampled the air and found mercury contamination in the school and neighboring buildings, which led to the decision to add human biomonitoring to the investigation. Fortunately, the public health laboratory already had a validated method to measure mercury in urine and ended up testing 30 people for mercury exposure. The laboratory determined most individuals did not have elevated above background levels of mercury. Those with elevated levels received referrals for specialized medical treatment.

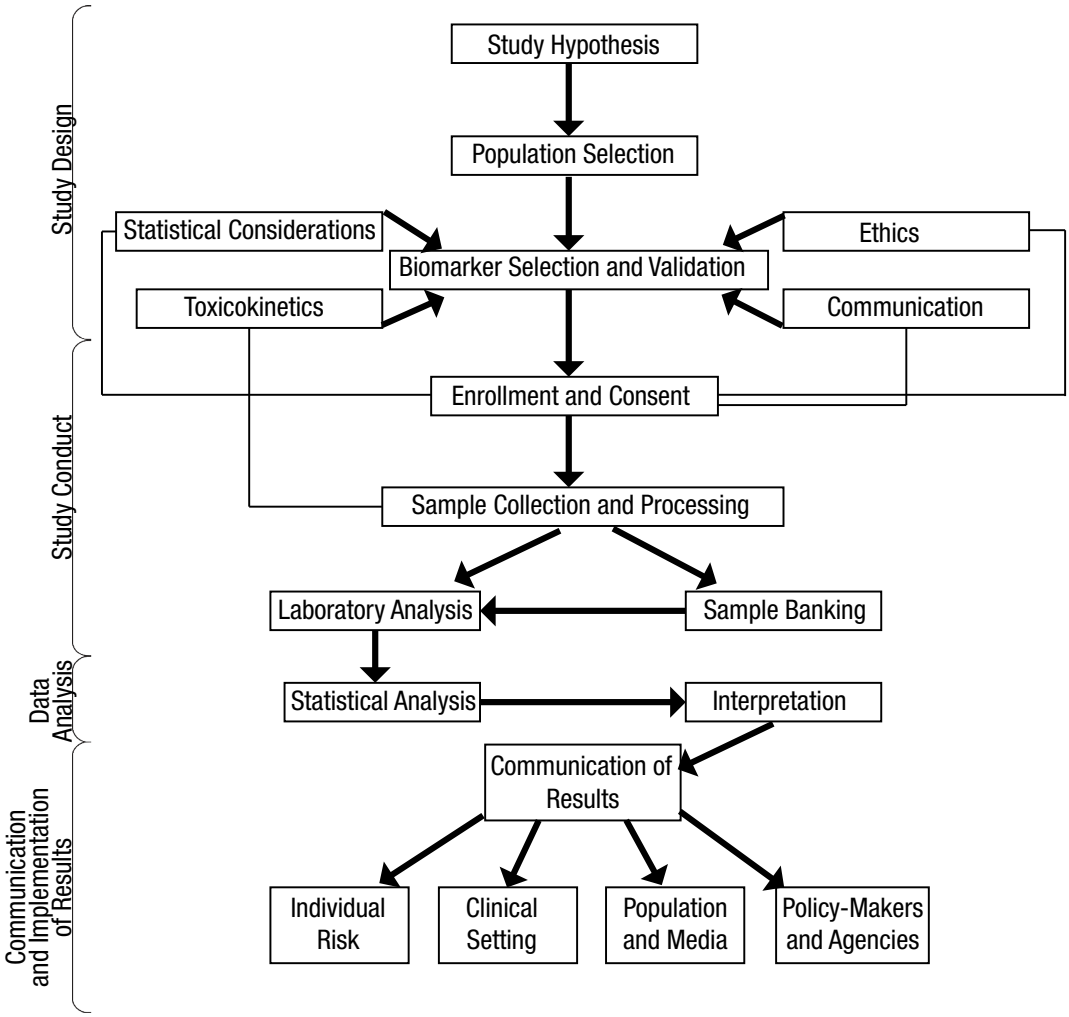
Disease Investigation or Rapid Response

Biomonitoring may be recommended in response to an exposure event to evaluate clinical measures in individuals and support diagnosis of poisonings and assessment of need for medical treatment. The investigation methods used would be similar to a disease outbreak investigation. Methods would typically require that exposed individuals be rapidly identified and referred to a clinical setting for specimen collection, diagnosis and follow-up by medical professionals. State health professionals would provide necessary laboratory or toxicology support. Epidemiologists may be involved to track outcomes over the course of the response, and to design follow-up investigations.

Stages of a Biomonitoring Study

The Committee on Human Biomonitoring for Environmental Toxicants at the National Research Council has developed the following algorithm to describe the essential stages of a biomonitoring study[3]. Laboratorians can see how their work informs and is informed by the broad expertise of many disciplines. It also points to the need for laboratorian expertise in each stage of a biomonitoring study.

FIGURE 1. HUMAN BIOMONITORING FOR ENVIRONMENTAL CHEMICALS



Stages of a Biomonitoring Study,
http://www.nap.edu/catalog/11700.html.

[3]National Research Council of the National Academies. Human Biomonitoring for Environmental Chemicals. Figure 4-1, page 86. National Academies Press, Washington DC, 2006

Multidisciplinary Team Needed for Biomonitoring

Biomonitoring is an inherently multi-disciplinary activity. Expertise in epidemiology, analytical chemistry, statistics and toxicology are necessary to develop a well-designed study, to identify and quantify chemical body burdens and to analyze study findings. Additional skills are needed to communicate and interpret biomonitoring information to individual participants as well as to the larger community. The most successful biomonitoring activities are those that involve collaboration among public health partners working to answer a defined environmental health question. This principle holds true regardless of the intended purpose of the biomonitoring activity: population-based surveillance, targeted exposure assessment, disease investigation or emergency response. Although for smaller, targeted studies one person can potentially play the role of several team members.

Epidemiologists design biomonitoring studies with input from colleagues in the other public health disciplines. The epidemiologist is responsible for overseeing the study design and monitors the overall execution of the study protocol throughout the duration of the study, usually as the principal investigator or as co-investigator. Ensuring daily adherence to protocols is a critical function of the epidemiologist for ensuring the validity of inferences drawn from the resulting data. As the leaders in design efforts, the epidemiologists determine the appropriate study type (longitudinal, cross-sectional, cohort, case-control, etc.), identify study populations, develop questionnaires, write protocols and recruit participants. Epidemiologists must collaborate with statisticians, chemists and toxicologists during the study design phase to develop a scientifically sound plan for the biomonitoring activity. Study implementation, data analysis and reporting of individual and aggregate data are also typically the responsibility of environmental epidemiologists.

Analytical chemists provide testing to measure the concentration of environmental chemicals or their metabolites in clinical specimens. These data may be used in conjunction with survey information to provide answers to environmental health questions.

Chemists assume responsibility for ensuring the quality of the analytical measurements for biomonitoring activities, without which the entire study may be called into question. Therefore, early inclusion of chemists in the study design is necessary. Analytical chemists provide written protocols for specimen collection, handling and transport. These issues are particularly important if the target biomarkers are ubiquitous environmental contaminants or environmental degradates (e.g., bisphenol A, triclosan, parabens). Chemists offer specific training to specimen collectors and in some instances may assist in the collection process. They work with toxicologists to identify the appropriate biomarker(s), considering not only the pharmacokinetics of the analyte(s), but also the feasibility of measurement. Chemists determine the appropriate analytical methodology for measuring the target biomarker(s) and write standard operating procedures. Chemists need to work with the study design team to develop a sample collection, processing and shipping schema to ensure valid samples are obtained for the laboratory analysis.

During the analytical portion of the study, chemists monitor multiple quality assurance indicators to verify the integrity of the data generated. Chemists may be responsible for reporting and interpreting findings along with appropriate reference ranges (if available) to environmental health professionals and/or clinicians.

Toxicologists determine the appropriate biomarkers for measurement after considering available environmental data and opportunities for exposure. Important factors which influence this determination include potential route(s) of exposure, duration of exposure (acute vs. chronic) and finally, metabolism, compartmentalization, and half-life of the analyte(s) within the body.

Biomarker selection is finalized after consultation with epidemiologists and chemists to assure practicality of specimen collection as well as the analytical capability and capacity of the laboratory. Toxicologists are also responsible for the determination of critical values and action levels. They play an active role in the analysis and interpretation of all study data to ensure that statistical inferences and conclusions are biologically plausible.

Statisticians provide valuable input in both the study design and data analysis phases of a biomonitoring activity. During the design period, statisticians calculate the minimum sample (population) size needed to adequately address the environmental health question. Following data collection, they recommend the appropriate metrics for evaluation of biomonitoring and survey instrument data. After laboratory results are reported, statisticians advise epidemiologists or perform data reduction and analysis of the study findings.

Physicians may provide medical oversight or consultation to an investigation, order clinical tests and most importantly, remain available to discuss individual results with participants. Physicians with a specialty in medical toxicology, occupational medicine or pediatrics, and those with training in epidemiology are especially helpful in interpreting medical risks and conveying that information to the patient. In biomonitoring studies that include biomarkers of effect, a measurable change in an organism that can be associated with a negative health outcome, physicians provide critical diagnostic information and analysis to the study.

Communications specialists provide essential expertise and support in communicating with a broad range of audiences about the status and progress of the study. According to the National Research Council (2006), communication of biomonitoring results may be the most vexing challenge to the field of biomonitoring. The uncertainties commonly associated with biomonitoring studies call for comprehensive expertise that enables combined application of risk communication and health communication principles and practices. Effective communication requires planning and capacity to develop and implement communication activities based on understanding of audience-specific needs. Communication plans also will vary by study goals, selected biomarkers and levels of certainty about health effects, exposure sources and prevention measures.

External Partners

Due to the complexity of biomonitoring investigations, drawing on the experience and expertise of a broad range of public health disciplines is advisable. In addition, there are non-public health partners whose input and collaboration will enhance the quality and strength of biomonitoring activities, such as environmental agencies, local industry groups and community groups.

Formal scientific advisory committees help guide important decisions during the planning and execution of a biomonitoring study, or for the duration of a surveillance program. An advisory committee that includes academic and/or industry scientists can help to increase scientific rigor and can strengthen the credibility of the overall process and results with the public and other key audiences. Additionally, including public citizens in the advisory committee can help with credibility and transparency during the design and planning phases. Appropriate budgeting and staffing is needed to support and facilitate effective advisory committees.

Laboratory Response Network

In 2003, the Laboratory Response Network (LRN) expanded its mission from only testing for biological threat agents to testing for chemical threat agents as well. The chemical component of the LRN, referred to as the LRN-C, worked to build a network capable of responding to chemical threat events over the past decade. The LRN-C consists of 62 public health laboratories throughout the US and its territories as well as a laboratory at CDC[4].

The 62 LRN-C laboratories have designated "levels" that correlate with their ability to perform certain tasks during emergency events. Level 3 laboratories work with hospitals and first responders to collect, package and ship clinical samples to other laboratories for testing. Level 2 laboratories test samples for toxic chemicals, metals and some chemical warfare agents. They are a state and local resource for responding to chemical exposure events, and some may assist neighboring states during large-scale events. Level 1 laboratories test for chemical warfare agents and serve as backup to CDC during large-scale emergencies.

The level 1 and 2 laboratories have the instrumentation, knowledge and personnel necessary to conduct most biomonitoring studies. This is an excellent resource available to states who wish to implement biomonitoring. States that may not have this capability can consult or partner with level 1 and level 2 states to initiate biomonitoring studies.

[4]http://emergency.cdc.gov/lrn/chemical.asp

Community Engagement

Most biomonitoring studies in public health settings will include some level of community engagement and public participation to provide an avenue for health departments and communities to exchange information, concerns, and resources. The laboratory is typically not responsible for this part of the study and may or may not be involved.

There are varying degrees of community and health department involvement, decision-making and control depending on the purpose, goals, and resources available for community engagement. These factors, combined with the considerable complexities and uncertainties around biomonitoring data, make it necessary to consider the multiple opportunities for community engagement throughout a biomonitoring study.

The following are basic goals for community engagement and public involvement:

1. Build public awareness and understanding of the biomonitoring effort as well as manage expectations;
2. Provide opportunities for members of the affected community and stakeholders to contribute to program design, implementation, and evaluation;
3. Achieve high participation rates within the target population(s) to be studied; and
4. Communicate biomonitoring results in a useful, effective and understandable manner.

Given the considerable complexities and uncertainties around biomonitoring data, it is necessary to consider the multiple opportunities for community engagement throughout a biomonitoring study. At each stage of a biomonitoring study, it is important to clearly articulate the goals of engagement in order to determine the best methods and set clear expectations for all involved, given the goals of the study and the resources available.

For many studies, citizen involvement is especially needed in early planning stages. Citizen involvement on advisory committees is a common method for gaining citizen input, support and participation. Local representatives and community members can provide important perspective on community concerns and values that can potentially impact the overall success of the study at any stage. This local knowledge and participation will aid in the design of the effort to ensure it simultaneously meets the needs of the community and the researcher or agency. Community members can be very helpful in communicating the goals and limitations of the project to others, encouraging participation and aiding in the success of communication efforts throughout the study. At the highest level of community-based participatory involvement, community members are enlisted as equal partners to join with investigators in designing the project and in decision-making throughout the conduct of the study.

SECTION I: NECESSARY LABORATORY INFRASTRUCTURE

Infrastructure

A laboratory considering implementing biomonitoring activities should ensure that there are adequate and appropriate laboratory facilities, equipment and information technology infrastructure to support the proposed activity. To begin, laboratories should follow the infrastructure guidelines developed to participate in the LRN-C, chemical response laboratory network. However, biomonitoring activities may require some additional considerations as described below. Laboratory design should include: adequate clean rooms, biosafety engineering controls, waste disposal (biological, chemicals and radiological), and specialized air-handling requirements for instrumentation.

Facilities

Biosafety and chemical safety considerations. Engineering controls serve as part of an overall Exposure Control Plan that describes the way risk is managed to minimize exposure to blood–borne pathogens and other organisms that might be present in the specimens. Engineering controls are design principles and the use of devices that will protect the workers from unnecessary exposure to pathogenic organisms. Elements of a biosafety program include controlled air flow (negative air pressure), the use of biosafety cabinets, splash shield, etc. Depending on the desired level of protection and the potential risk, various levels of biosafety may be needed, from biosafety level 1 (BSL-1) to BSL-4 (reserved for work with the most dangerous pathogens). Most of the biomonitoring work will be conducted in BSL-2 laboratory space.

Equally important is minimizing workers' exposure to chemical substances and solvents. The use of chemical hoods, proper chemical storage and signage are only some of the elements of an effective chemical safety plan. It is recommended for all personnel working on biomonitoring studies to reference their laboratory's chemical hygiene plan as well as to follow universal precautions when working with biospecimens. Additional information can be found in the Occupation Safety and Health Administration's standards related to laboratories[5] and CDC's *Biosafety in Microbiological and Biomedical Laboratories 5th Edition*[6].

Clean rooms may be necessary to minimize contamination background in blanks, standards, quality control materials and study specimens. This is especially important when measuring elements and chemical compounds of interest at very low concentrations in human tissues. For example, Class 100 to 10,000/1000 clean rooms have been used effectively for controlling contamination for analysis of trace elements by Inductively-Coupled Plasma Mass Spectrometry

[5]http://www.osha.gov/SLTC/laboratories/index.html
[6]http://www.cdc.gov/biosafety/publications/bmbl5/

(ICP-MS). Whether a Class 100 or 1000 or 10,000 clean room is necessary depends on what level of background contamination can be tolerated. Some laboratories have a Class room 100 for preparation of specimens, standards, and blanks, then transport prepared specimens to an instrument for analysis residing in a Class 10,000 or uncontrolled laboratory setting.

Waste disposal must also be a part of any laboratory design. Special consideration should be given to the handling of mixed waste and protocols for handling radioactive materials. Suitable containers should be made available along with training on how to properly dispose of waste and to manifest the waste for proper disposal in accordance with state and federal laws.

Backup power, such as an emergency generator bridged with an uninterrupted power supply (UPS) system, is ideal to assure continued instrument operation. If no emergency power is available, at least consider a UPS system to allow a suitable time for the instrument to be powered down without being subject to potentially damaging power surges and other severe problems that might occur from a power failure.

Given the nature of the specimens being handled and the implications of the investigations, laboratory and data security must be addressed. Individual laboratories will need to determine the appropriate level of security for their application. At a minimum, security should include restricted access to all laboratory and survey data, specimens and laboratory facilities. These restrictions may be accomplished in a combination of ways including:

- Technology-based methods: proximity card access, biometric fingerprint or retinal readers, electronic surveillance and alarms
- Policies and procedures: chain of custody, inventory control, data privacy.
  Data privacy issues should be part of the initial project planning and are discussed in more detail in Section II: Biomonitoring Study Design.

Equipment

Analytical instrumentation used in biomonitoring must be sufficiently sensitive and specific to detect the analyte of interest in human tissues such as blood and urine. The instruments chosen should provide the best sensitivity possible within reasonable cost to assure that low detection limits can be attained. The following guidelines may be used as a starting point for selecting the appropriate instrumentation/technology for a given analyte or analyte class. The platforms discussed in this section are recommendations and this is not an all-inclusive list. Alternative instrumentation that meets your laboratory's performance standards should be used, as appropriate, for the intended purpose(s) of the study. Also, as newer technology is developed, it may be better suited for biomonitoring studies.

Trace elements, including some radionuclides, may be determined using ICP-MS. Laboratories who are Level 1 or 2 in the LRN Chemical Terrorism Response Network already have an ICP-MS instrument that can be used for biomonitoring. ICP-MS instruments require argon as the plasma

gas and other gases such as hydrogen, helium or ammonia, as needed, for collision cell applications. Speciation for elements such as arsenic may also be desirable. Speciation will require a high-performance liquid chromatography (HPLC) system and associated software.

Volatile organic compounds and metabolites may require gas chromatography – mass spectrometry (GC/MS) or gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis.

Semi-volatile and non-volatile compounds and metabolites that are soluble in more polar solvent mobile phase can be analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS.) CDC/NCEH/DLS has developed some methods for these classes of compounds; these are outlined in the testing portion of this guidance document.

When new instrumentation must be purchased, consideration should be given to the instrumentation (vendor, model type) used by existing biomonitoring laboratories to facilitate the best success at bringing methods up and validating them in a reasonable amount of time. As with any instrumentation, service contracts should be considered past the duration of a standard one-year warranty to assure the instrument is well maintained and can be brought online again quickly if a problem occurs.

Commonly-used **clinical chemistry equipment** will also be needed, such as: centrifuges, shakers, vortex equipment, autodilutors and other similar equipment found in a clinical laboratory. Adequate refrigerator and freezer capacity is required for the storage of standards, isotopes and specimens (see Storage Section). Biomonitoring measurements are commonly corrected for urine dilution by specific gravity or creatinine correction. Specific gravity is determined using a relatively inexpensive refractometer. NHANES reports creatinine corrected values. An in-house or vendor capability for urine creatinine analysis is necessary to match the published CDC data. Organic measurements in blood/serum may be normalized for lipid content.

Sample preparation equipment such as microwave digestion and hot blocks may be necessary for elemental determinations. Solid phase extraction and more traditional extraction techniques should be available for organic analyses.

Information Technology

Once thought of as a support function, the delivery of laboratory IT services has now evolved to the point where electronic recordkeeping and automated data management are mission-critical components of public laboratory operations. Modern analytical instruments include complex computer systems to collect and evaluate data. Interfaces are needed to transmit these data to a laboratory information management system (LIMS) for a centralized repository of specimen data and associated management, tracking, analysis and reporting functions. Information technology, both hardware and software, are an integral part of instrumentation and data processing required for the analyses of biomonitoring specimens. Indeed, acquisition of IT equipment, support function, and technology systems are often among the largest expenditures a laboratory makes. See Appendix 3 for more general information on LIMS.

Laboratory Personnel

While hiring and retaining talented laboratory personnel is of paramount importance in any laboratory, staffing a biomonitoring laboratory presents a set of unique challenges. The technology and instrumentation used for biomonitoring testing has a lot in common with environmental testing laboratories, so training in environmental testing may be helpful. Staff must also be trained to safely handle potentially infectious clinical specimens and work with challenging new matrices. Additionally, as test results may be used for diagnostic purposes (as opposed to research only), the laboratory and laboratory personnel must meet CMS requirements for clinical laboratories, commonly known as CLIA requirements[7]. CLIA requirements differ based on the type of testing provided. Virtually all biomonitoring labs would fall under the "high complexity" category and "toxicology" subcategory.

The following list includes recommendations for staff qualifications, taking into account CLIA requirements.

Educational background

Laboratory personnel must be appropriately qualified to perform high complexity testing under CLIA '88 regulations. Analysts should have a B.S. or B.A. in chemistry, biology or related scientific field or appropriate amount of course-work required for high complexity testing required by CLIA '88. Personnel should have a minimum of six months experience in the field of testing which they are performing.

Computer proficiency

Personnel should be proficient with Laboratory Information Systems (LIMS) and be familiar with statistics calculations and spreadsheet software such as Microsoft Excel.

Training and professional development

Laboratory personnel will also need the following additional on-the-job training and continuing professional development over time.

Instrumentation training is necessary for GC-MS, LC-MS, LC-MS/MS, ICP-MS and other related platforms necessary to test human tissues for analytes of concern. Formal training may be provided by the instrument vendor or by an analyst proficient in the use of the instrumentation. CDC offers analyte-specific training which includes some instrumental component. These trainings are given in various formats including hands-on at CDC or through computer- or web-based training. Training on site-specific methods and procedures should be provided internally by each laboratory.

[7]CLIA regulations may be found at: http://wwwn.cdc.gov/clia/regs/toc.aspx

Analytical expertise must be continually updated. Personnel should have a basic understanding and knowledge of analytical chemistry principles and be capable of verifying and validating test methods. Analytical accuracy and precision, selectivity and specificity are examples of necessary knowledge. Laboratory personnel should participate in continuing education related to analytical chemistry, toxicology, and epidemiology. This may be accomplished through attending conferences, vendor presentations, webinars, etc.

Human subject protection and data confidentiality training is needed for most studies involving clinical specimens. Laboratories must fulfill requirements for data privacy training and adhere to policies and practices for approval of studies involving human subjects (Institutional Review Board and/or Human Subjects Review).

Completion of the certification course offered by the National Institutes of Health (NIH) Office of Extramural Research for Protecting Human Research Participants is recommended for all investigators responsible for biomonitoring study design and execution, and is required by law for any study conducted with or supported by federal funds.

Safety training in both chemical and biological safety is required based on individual laboratory requirements and federal regulations. At a minimum, blood-borne pathogens training, hazardous waste disposal and chemical safety training are required. Familiarity with OSHA regulations and good laboratory practices are necessary. Radiation safety training is required if applicable.

Interdisciplinary training in statistics, epidemiology, and toxicology and risk communication is desirable. Specialized training in health risk communication is recommended for staff engaging with community members and the media.

Ongoing worker safety program

Given the interaction with human biological samples, worker safety requires special precautions. Appropriate immunizations (e.g., Hepatitis B for work with blood) should be offered based on each laboratory's policy and exposure control plans. Required personal protective equipment (PPE) should be provided such as gloves, face shields, etc. Exposure control measures such as Biosafety Cabinets, splash shields, BSL2 laboratories should be utilized where needed. More information on biosafety can be found in CDC's *Biosafety in Microbiological and Biomedical Laboratories 5th Edition*[8].

Additionally, laboratories working with chemical hazards should be aware of ongoing safety issues and attend regular worker safety trainings as required by their state. Laboratorians should always use fume hoods when working with chemicals as well as standard personal protective equipment.

[8]http://www.cdc.gov/biosafety/publications/bmbl5/

SECTION II: BIOMONITORING STUDY DESIGN

Overview

Biomonitoring studies of human populations, whether conducted as public health surveillance or investigation, require collaboration among public health professionals with training and experience in designing and conducting observational studies of human population health including epidemiology, analytical chemistry, toxicology and statistics. Flaws in the study design or failure to adhere to study protocols can compromise the validity of the data. Sufficient resources (time and staffing) should be budgeted to ensure a scientifically rigorous study design prior to starting a biomonitoring activity. Community engagement or participation in the study design process is strongly encouraged and requires additional time and resources to coordinate.

Key components to be included in the study design are:

- population selection,
- statistical considerations (such as sample size) and data analysis methods,
- population sampling strategies,
- enrollment and consent methods,
- biomarker/matrix combination selection,
- methods for collection and processing of specimens and transport to the laboratory,
- training of field staff,
- analytical methodology,
- methods for interpreting results, and
- communication plans.

Each of these steps should be clearly described in a written study protocol document. Additionally, it will always be necessary to ensure human subjects protection, adherence to data privacy protection and good ethical practices. The end of this section will address these latter practices.

Study Protocol Development

Just as good laboratory work starts with clearly written laboratory protocols that provide quality assurance and control, conduct of human health studies begins with detailed written protocols covering every aspect of the study design. Protocols are written to provide a reference document that will guide conduct of the study (which can often go over several years) and will ensure that key elements in the design are maintained. The project epidemiologist will carefully monitor adherence to study protocol and will document protocol or procedural changes that can occur during the study. This is necessary because changes in participant recruitment and data collection protocols have the potential to significantly affect the interpretation and validity of the results. Following is a brief description of the primary protocols needed for quality management in a biomonitoring study.

Participant selection and recruitment protocols describe the specific methods to be used by study staff for sampling and selecting participants from a target population, making contact with participants (by phone, mail, or other means), and obtaining informed consent. Recruitment protocols include methods for tracking the number of people who are eligible and consent to participate, as well as non-participants, people in the study population who are determined to be ineligible, refuse to participate, or unable to contact. Documentation of selection and recruitment protocols (and any changes in protocols) is important to the validity of inferences that are drawn from the study results. Protocols will include attachments of study materials such as letters to participants, informed consent documents, questionnaires and other data collection instruments. Protocols should be in compliance with the latest standards for data collection on race, ethnicity, sex, primary language and disability status. Current standards can be found online[9].

Specimen collection protocols provide detailed step-by-step instructions regarding how, where and when specimens are to be collected, processed, preserved and transported to the lab. Laboratory personnel work with the epidemiologist to write procedures for field staff and instructions for participants on specimen collection. In some situations, laboratory personnel may train field staff in specialized specimen collection procedures to ensure the integrity of the sample for testing. This is particularly important if the target biomarkers are ubiquitous environmental contaminants or environmental degradates (e.g., bisphenol A, triclosan, parabens).

Specimen storage and handling protocols must be included as part of the overall study protocol documents. Unless specimens are being stored for other uses, procedures for when and how specimens will be destroyed at the end of the study are also needed. Conditions and length of storage time should be documented. It is strongly recommended to include field blank samples (e.g., high-purity solvent(s) placed in a sample container and processed as a biological specimen) in the protocols for the collection and/or processing of biological specimens for programs/studies with a current or potential biomonitoring component.

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[9]Office of Minority Health. Final data collection standards for race, ethnicity, primary language, sex, and disability status required by section 4302 of the Affordable Care Act. US Dept of Health and Human Services Web site. http://minorityhealth.hhs.gov/templates/browse.aspx?lvl=2&lvlid=208

Data security protocols describe methods for ensuring data security and privacy protection, data cleaning and aggregation analysis methods, and define the specific study outcomes (e.g., geometric mean, standard deviations, and percentiles).

Participant protection protocols identify and address any legal or ethical issues, and describe methods for minimizing privacy risks to participants, especially among vulnerable groups.

Reporting and communications protocols address plans for interpreting and communicating results with multiple audiences including participants, community members, legislators, media representatives, and medical providers. Reference populations or health-based reference values should be specified for the interpretation of findings. A protocol for referral to medical follow-up should also be specified. Analytes with a known medical endpoint (e.g., lead) must have a timely reporting protocol to meet clinical guidelines.

Population Selection and Study Sample

There are a number of important considerations in the identification and selection of a study population including the purpose of the study, the type of chemical exposure that is of interest (the exposure scenario), and whether biomonitoring will address past or only current exposures. The sampling criteria should include a description of the following items.

Target population: The sample should provide a frame of reference for the population of concern. Ideally, the study population should be representative of the target population by age, sex, race/ethnicity, or any other characteristics considered to be important.

Selection methodology: The extent to which findings from the study population can be generalized to the general population will be dependent on how the study population is selected–whether the study population is self-selected or participants are selected based on specific characteristics or features. Additionally, the degree of non-participation will influence the ability to generalize to the general population.

Random sampling within the defined target population is the preferred method for selecting participants in order to minimize selection bias. Criteria for recruiting and selecting participants should be clearly identified. Convenience sampling can result in selection bias and, therefore, data may not be generalizable to a larger population. When convenience sampling is used, clearly describe characterization of the sample, so misinterpretations will be less likely.

Matching can improve internal validity of study results. This involves selecting one or two non-exposed or non-diseased individuals for every exposed or diseased one. Matching typically involves selecting individuals who are similar in all relevant characteristics (such as age, sex, and race/ethnicity), differing only by whether they are exposed or diseased.

Sample size: If comparisons are to be made between populations, consider the sample size needed to be able to achieve adequate statistical power to allow for detection of meaningful differences in the outcome. Generally, the smaller the group, the larger the variation due to inter-individual differences, thus, the more uncertain the results.

Epidemiologic Study Design

The purpose of the study determines the most appropriate study design. Brief explanations of the study purpose for various study design options are provided below.

Surveillance is the ongoing systematic collection of data of interest to detect changes over time. Specifically, biomonitoring may be conducted for the purpose of detecting and measuring spatial or temporal differences in population exposure. Biomonitoring as public health surveillance can also be used to evaluate the efficacy of public health actions to reduce exposure. Two primary designs are used:

- A *cross-sectional sample* of a defined population is the design most often used in surveillance studies.
- A *cohort design*, in which the same individuals are followed over time, may be used at the state or local level. It is less common due the greater expense and losses to follow-up that can compromise validity of the results.

Targeted investigation is used to observe the course or the interaction (if there is one) between chemicals or interventions and health outcomes. These investigations are generally conducted in response to the discovery of environmental contamination or a cluster of disease with a possible chemical origin to measure the range and distribution of the exposures in the community, to determine whether exposures are elevated above a reference level, and to recommend public health action. Possible study designs include:

- Ecologic study: examines the relationship between exposure and outcome at the population-level.
- Cross-sectional study: investigates the relationship between exposure and disease prevalence in a defined population at a single point in time.
- Case-control study: analyzes multiple exposures in relation to a disease; study participants are defined as cases and controls, and their exposure histories are compared.
- Cohort study: assesses multiple health effects linked to an exposure; subjects are defined according to their exposure levels and followed for disease occurrence.

Note: A convenience sample can be used if the goal is to describe only the individuals being sampled. Convenience samples cannot be used to make valid statistical inferences or to generalize the results to a larger population.

Investigator-initiated research is used to test a proposed hypothesis related to sources of exposure or health outcomes. In this case, the design will depend on the hypothesis under study. These studies involve collecting environmental or health data, in addition to biomonitoring data. Ecological and cross-sectional studies are not recommended for measuring causal relationships. Cohort or case-control studies are preferred for testing etiological hypotheses.

Exploratory studies involve biomonitoring in a population, typically with no known exposure or only minimal exposure, for exploratory purposes or to establish reference levels against which biomonitoring results in other "test" populations will be compared. When establishing a reference range, comparability between the reference and the test population is most important. A cross-sectional study design is recommended. The population should be inclusive, representative and well-characterized by host and demographic information (age, sex, race/ethnicity) to allow for valid comparisons. Environmental monitoring data may be needed to confirm that the reference population has minimal or no exposure, or to help inform the results.

Statistical Analysis Plan

A statistical analysis plan of biomonitoring results in aggregate is an essential component of the overall study design and is included in the study protocol document. Appropriate statistical methods should be described for each descriptive analysis (characterizing the study population) and for each hypothesis to be tested.

Obtaining statistical consultation during the planning of the study is recommended for a well-executed statistical analysis plan. The plan should include a clear statement of:

- dependent variables, independent variables and covariates;
- analytical outcomes, statistical models, sampling frame, sample size calculations;
- test methods for determining variable distributions, central tendency and variability;
- comparisons by pre-determined strata.

Potential sources of measurement error, selection bias, and confounders should also be identified. A plan for minimizing and controlling such errors is recommended to strengthen the validity of the results. Geographic trends may need to be included in study design and the sampling plan should ensure achieving adequate sample size for meaningful analysis.

In addition to the sampling methods discussed previously, other measures that can be taken to avoid biased results include:

- Use same/similar sources and procedures for the groups being compared;
- Mask investigators to the exposure status of a subject so that they make unbiased decisions when assessing the outcomes (or vice versa depending on the study design);
- Identify clearly what measures are used for defining exposure and outcome/disease for both accuracy and comparability.

Statistical Analysis of data is done in three major steps: data preparation, descriptive analysis, and inferential analysis. Laboratories should work with epidemiologists or statisticians to ensure they have robust data. Appendix 2 has more information on statistical analysis.

Data Quality Assurance and Control

The data analysis plan should include specific, data-related quality assurance/control measures for all data collection procedures. This includes protocols for handling data for:

- assuring protection of confidential personal identifiers,
- recording and keeping track of data from multiple sources,
- checking for completeness and accuracy of data collection/abstraction,
- developing a data element dictionary.

Additionally, the plan should describe what information (demographics, medical history, biological samples, etc.) is collected, its sources, which data collection methods are used, and how these methods were validated.

Protection of Human Research Participants

With all studies that involve human subjects, the lead investigator is responsible for knowledge of and compliance with state and federal laws that protect the rights of participants in health research. Information about the training, ethical guidelines and federal laws governing human subjects can be found on multiple websites[10,11]. In all human research studies, Institutional Review Board (IRB) review will be necessary which requires adherence to highly specific practices related to human subjects protection, data privacy protection and ethical practices[12,13,14]. Investigators are strongly advised to consult with their IRB for a determination of whether a given project is deemed to be research or public health practice, as this determination is subject to interpretation. A brief outline of key considerations follows.

Informed consent should be obtained from all participants, as most biomonitoring activities require information sharing between the investigator and the prospective participants. If secondary use of specimens or follow-up with participants is anticipated, consent documents should provide opportunity for participants to give permission for future contact and continued storage and use of specimens beyond the study period.

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[10]http://grants.nih.gov/grants/policy/hs/ethical_guidelines.htm

[11]Federal law CFR Title 45, Part 46 governing human subjects protection can be found at http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm

[12]The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report: ethical guidelines for the protection of human subjects. Washington (DC): U.S. Department of Health, Education and Welfare; 1979

[13]Institute of Medicine. Preserving public trust. Accreditation and human research participant protection programs. Washington (DC); National Academies Press; 2001.

[14]National Bioethics Advisory Commission. Ethical and policy issues in research involving human participants [Internet}. Bethesda, MD: National Bioethics Advisory Commission; 2001 Aug [10/19/2011]. Available from: URL: http://bioethics.gov/

Risks and benefits of biomonitoring for participants should be clearly identified and explained to the individual so that he or she can make an informed choice. Risks generally refer to individual health risks such as the potential for injury, infection or pain from an invasive procedure. But other risks, such as the potential for heightened anxiety from a procedure, discomfort, inconvenience and cost are often included. Benefits should clearly state how the individual participant benefits in terms of opportunity for medical treatment, counseling or follow-up to identify and remove sources of exposure. Incentives (monetary or gifts) should be described in the protocol but generally are not considered benefits of participation.

Protection of data privacy is paramount. With few exceptions, biomonitoring necessarily requires the collection of individually-identifiable health information that will be classified as private or confidential. All staff that requires access to private data should be trained on the laws governing the protection of data privacy and data practices that are applicable in their jurisdiction. Standard protocols should include password-protected systems on all computers and locked cabinets for maintaining files. State laws generally include severe penalties for release of private data on individuals. Participants should receive absolute assurance of the protection of their individual health information and that no identifying information is ever released in reports, publications, or other communications.

De-identification of specimens in the laboratory is often recommended to protect privacy. In de-identification, personal identifiers are maintained by investigators in a database and linked by an individual study identification code to the laboratory results. However, anonymization of specimens—identifiers are permanently severed from all information about the source of the specimen and results cannot be traced back to the individual—is not appropriate for biomonitoring in a public health context. Anonymization limits the use of the data for understanding health effects, prevents informing individuals of their results and prevents intervention that could potentially protect individuals at risk-all fundamental goals of public health practice.

If a sample is determined to require chain of custody, the Sample Collector must initiate the process. More information about such procedures as well as a sample chain of custody form are in Appendix 5.

Other Ethical Considerations

There are important ethical considerations with respect to biomonitoring beyond the standard protection of individual human subjects. Biomonitoring of community exposures has led to discussions of the rights of communities as research subjects[15]. In study planning with the community, investigators will need to incorporate community values, and consider how the project may potentially harm or benefit the community as a whole. The potential for unintended economic, social and political consequences to the community should be addressed.

[15]Blumenthal DS. A community coalition board creates a set of values for community-based research. Prev Chronic Dis. V.3 (1). Available from: URL: http://www.cdc.gov/pcd/issues/2006/jan/05_0068.htm

SECTION III: BIOMARKER SELECTION

The decision regarding which substance and biomarker will be investigated should be made early in the study design, as it greatly influences the selection of the specimen type to be collected. Laboratory scientists and chemists should work closely with epidemiologists and toxicologists to determine the study biomarker(s); their scientific input and understanding is essential at this phase. Several variables need to be thoughtfully considered prior to reaching a decision including: the purpose of the study, the properties of the chemical of interest, biomarker specificity, available analytic methods, and feasibility.

The most common clinical matrices are urine, blood, serum, and saliva. Other matrices have also been used such as hair, nails, breast milk and adipose tissue, as necessary, to measure the analyte of interest; but these other clinical matrices must be approached with caution, since the interpretation of the analytical results can be challenging. There may be special analysis techniques or specimen handling for the less common biospecimens. It is also important to understand the potential effects of the biological matrix on the concentrations of the target biomarkers.

Purpose: It is important to consider the purpose of the biomonitoring activity, whether the study intent is to investigate a single question or to gather surveillance information for a given population. If the purpose of biomarker collection is to link internal body burden to an adverse health effect, investigators must select biomarkers that are specific to the health outcome of interest. One must also consider whether the biomarker has been measured at the appropriate time, the critical life stage of interest, and the disease's induction and latency characteristics. Some chemicals have biological half-lives measured in years, which makes it feasible to detect exposures long after they occurred.

Properties of the chemical and pharmacokinetics: Investigators must understand the properties of the chemical of interest and factors governing the absorption, distribution, metabolism and excretion of the analyte. Investigators must research potential routes of exposure, with respect to differences in uptake, which may affect storage and pharmacokinetics in the body making one specimen type preferable over another. Rates of analyte metabolism may also influence specimen selection. For example, some organic compounds are readily metabolized and, therefore, it may be preferable to measure the metabolite in urine rather than the parent compound in blood, as it allows a longer time period for specimen collection[16]. As a very general rule, persistent chemicals are generally measured in blood, while non-persistent chemicals (or their metabolites) are measured in urine.

[16]National Research Council of the National Academies. Human Biomonitoring for Environmental Chemicals. National Academies Press, Washington DC, 2006

Depending upon the pharmacokinetics of the compound, a sample might represent exposures that occurred yesterday, in the past month or in the past decade. Substances with short half-lives may not be feasible to measure unless the timing of exposure is known and recent, or if the exposure is frequent or ongoing such that the biomarker will likely be continuously present. It is also useful to understand inter-individual variability in pharmacokinetics of the chemical and to collect any such information that may impact biomarker concentration (e.g., age, body build, health status, concurrent exposures). Information on how concentrations vary over time within the same individual is also important, especially for one-time biomarker collection. The selected biomarker and media should adequately reflect body burden and if not, additional metabolites and/or specimens in alternative media should be collected.

Biomarker specificity

The biomarker should be specific to the chemical(s) of interest, particularly if one of the intentions is to elucidate potential pathways and sources of exposure. Certain metabolites may be common to several parent compounds. For example, 3-phenoxybenzoic acid is a metabolite common to several pyrethroid insecticides, some of which are more commonly found on food while others are usually found in residential settings. Further, certain metabolites may also be environmental degradates which can obfuscate interpretation of results. For example, the environmental degradation of the insecticides chlorpyrifos and chlorpyrifos-methyl results in the formation of the same chemicals as does human metabolism. Therefore, by measuring the metabolite 3,5,6-trichloro-2-pyridinol (TCPy) in urine, one cannot distinguish whether exposure was to chlorpyrifos or chlorpyrifos-methyl or to TCPy itself.

Analytic specificity and sensitivity

Investigators must evaluate available analytical methods to determine the robustness of the method, and whether its detection limits will result in data suitable to answer the study questions. The limit of detection may be more of an issue for evaluating “background” or “environmental” levels of a biomarker compared to levels anticipated to occur from occupational exposure or community exposure to a known point source of contamination. The sensitivity, specificity, and potential for false-positive or false-negative results must be considered.

Feasibility must factor into selection of biomarkers. Analytic costs, laboratory precision and accuracy, ease of collection, invasiveness, the requisite volume of sample needed for analysis and the stability of the compound are all factors in assessing feasibility.

While theoretically any tissue type can be used for biomonitoring purposes, in practice, the ease and non-invasiveness of obtaining a specimen is a major consideration in study design. For these reasons, whole blood, serum and urine are used most commonly. These matrices may be collected in a relatively non-invasive manner while still providing specimens appropriate for a measurement of a wide range of organic and inorganic moieties. Less common but sometimes

used specimens include hair, fingernails, breast milk, adipose and other tissues. While hair and fingernails may initially appear to be good choices, they are prone to exogenous contamination and may require tedious sample cleanup to be viable. In addition, there are no validated methods for quantitative analysis nor are there any reference values. The remaining sample types are far less easy to obtain from study participants, each presenting their own unique analytical challenges. As a practical consideration, non-invasively collected matrices typically result in significantly reduced collection costs and increased participation rates.

Investigators should consider these two critical questions: Does the laboratory have the capability, analytical instrumentation, necessary skills and capacity to perform the method in the allotted time frame? Will the data generated be directly comparable to those in the literature or will additional post-analysis calculation be required? The laboratory must demonstrate method validation to address these questions and provide pilot data before initiating a study.

Correction for Urine Dilution

Some clinical measurements, such as analytes measured in urine, more accurately reflect an individual's internal dose when normalized.

Analyte measurements (trace metals, parent compounds and metabolites) in urine can vary significantly depending on the hydration status of the donor, i.e., how dilute or concentrated the urine sample is. A common way to account for that variability is to correct the analyte concentration for the amount of creatinine measured in the same sample. Creatinine is a normal breakdown product of muscle creatinine which is filtered from blood by the kidneys and excreted in urine. It serves as a good indicator of kidney function and urine strength. Final test results are typically reported as micrograms (ug) analyte/gram (g) creatinine.

Creatinine correction is particularly important when collecting the "spot" urine samples frequently used for biomonitoring, as these specimens have greater variability in strength as compared to a 24-hour urine collection. Generating the correction factors requires separate analytical procedures (in compliance with all respective laboratory Quality Assurance standards) run on each of the participant specimens–in effect, an additional biomonitoring analyte. In the absence of in-house capability, the methods can be contracted to clinical laboratory services.

When collecting serial samples from an exposed individual to monitor analyte excretion, creatinine correction is critical, as dehydration and/or the administration of intravenous fluid therapy will greatly influence the concentration of the target analyte in urine. By normalizing the data for creatinine, the hydration variable is eliminated and analyte concentrations can be more accurately evaluated over time.

Equation for correction: ug analyte/L x dL/ mg creatinine X 100 = ug analyte/ g creatinine.

Additional references may provide more information on creatinine correction[17,18]. Besides creatinine, other methods used for adjusting urinary dilution are specific gravity and osmolality. Specific gravity can be easily measured using a hand-held refractometer, which is calibrated with deionized water prior to each measurement.

Interpretation of creatinine-corrected results should also recognize that creatinine correction can also partially adjust for differences in lean body mass or renal function among persons.

Lipid Adjustment

Certain analytes are concentrated in the lipid fraction of serum, so lipid adjustment of results is recommended (reported per gram of total lipid). Lipid adjustment of the results better reflects the amount stored in body fat. Examples of serum analytes that are often lipid adjusted include poly-brominated diphenyl ethers (PBDEs), organochlorine pesticides, polychlorinated biphenyls (PCBs), and dioxins. Of note, however, serum results also may be reported per whole weight of serum to allow comparison with studies that report levels using these units.

[17]http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf, pages 5-6.

[18]http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info:doi/10.1289/ehp.7337

SECTION IV: ANALYTICAL PROTOCOL AND METHODOLOGY

The overall reliability of analytical testing depends on a quality management system that ensures proper design and implementation of a series of steps including: biological specimen collection, sample pretreatment, extraction, clean-up, concentration and instrumental measurement. Analytical method validation confirms that the method is suitable to detect, identify and measure (both accurately and precisely) the target compounds, thus verifying and quantifying method performance. While analytical method validation or verification is a concept applied in all areas of chemical measurements, biomonitoring applications present many unique challenges highlighted in the following sections.

In general, biomonitoring involves measurement of very low levels (e.g., parts per billion), often near the limit of detection. External contamination is a critical issue, and sources of external contamination deserve special discussion. For example, materials to draw blood or specimen containers may also contain the analyte of interest. Materials used in the lab (paper towels, hand soaps, etc) may contain the chemical of interest and result in contamination; pesticides sprayed outside the laboratory building may be tracked into the labs, volatilize, and result in contamination; exposing serum to air by repeatedly opening the vial may introduce PBDE-containing dust and falsely elevate results. These are a few examples that have caused measurement inaccuracies. For a laboratory doing biomonitoring measurements, it is imperative to consider such sources of external contamination—whereas this is not a consideration (or not to the same extent) for other clinical labs. Pre-screening of materials may be necessary.

Quality Management System

Every biomonitoring study must have a quality management system, which ensures the integrity of the samples, the analyses and the data produced. CLIA requirements (outlined in Section V: Results Reporting) provide specific guidance for laboratories. For the analytical portion of the investigation, this includes written Standard Operating Procedures (SOPs) for specimen collection, handling and transport; sample processing, sample analysis, and quality control. Laboratory methods must be internally validated including determination of method accuracy and precision. Chemists must be thoroughly trained in all aspects of the procedures prior to sample analysis. Minimally, each study should have quality control specimens that are analyzed concurrently with study samples as well as external assessment of laboratory proficiency.

Initial Considerations in Analytical Method Selection

A number of critical considerations must be taken into account when selecting the analytical method:

- What biomarker has been selected?
- What matrix is required?
- What analyte(s) need to be assayed? What range or concentration will be evaluated?
- What detection limit needs to be achieved?
- How will the data generated from this analysis be used? Will it be used for trend data, identification, or quantification? This will determine if you use a quantitative, semi-quantitative or qualitative method.
- What instruments/methods are currently available?
- Any special criteria that need to be met with respect to sample size, processing, storage or/preservation, etc?
- Are there appropriate analytical standards, controls and proficiency programs available?

Sources of biomonitoring methods

For laboratories initiating a biomonitoring project, there are several resources to find published laboratory methods. CDC's *Fourth National Report on Human Exposure to Environmental Chemicals* includes an appendix with a list of peer-reviewed methods[19] and also lists other peer-reviewed biomonitoring articles on their website[20]. Other peer-reviewed publications can be found using search engines such as the National Center for Biotechnology Information[21]. CDC houses some methods on the LRN-C website, a member-only site which has a detailed list of SOPs for methods within the network. Additionally, APHL maintains a Biomonitoring Database[22] where member laboratories input and update their current biomonitoring information, including methods. If methods are not posted, contact information is available to request method assistance, and a discussion board allows free-flowing conversations among the laboratories.

Specimen Collection

Once investigators have ensured that the proposed specimen type appropriately reflects the body burden of the biomarker there are a number of considerations in biological specimen collection: containers and tubes, specimen identification and documentation, collection method, shipping, storage and banking. Specimen collection protocols will provide detailed step-by-step instructions and describe how, where and when specimens are to be collected and transported to the lab. Laboratory personnel should work with the epidemiologist in writing procedures for field staff and instructions for participants on specimen collection. In some situations, laboratory personnel may train field staff in specialized specimen collection procedures to ensure the integrity of the sample for testing.

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[19]http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf, Appendix C: References for Biomonitoring Analytical Methods, page 507.
[20]http://www.cdc.gov/exposurereport/biomonitoring_articles.html
[21]http://www.ncbi.nlm.nih.gov/
[22]http://www.aphl.org/aphlprograms/eh/biomonitoringdatabase/Pages/default.aspx

Specimen containers/tubes used for biomonitoring

Potential contamination of the containers can introduce a bias in laboratory measurement. The suitability of a given lot of collection tubes must be assured by the laboratory prior to the collection of specimens. This is particularly important when the laboratory is measuring a chemical or metabolite common in the environment or that could potentially leach from specimen containers.

For trace elements, such as lead or mercury, some tubes are commercially available from lab supply distributors used by clinical/medical laboratories. Some specimen tubes are marketed specifically for trace metal analysis and might come with a certificate indicating that they are relatively "metals free." If a certificate is not available, or if the levels indicated are higher than those measured in the study, additional quality control steps must be taken.

With the exception of trace elements, however, the analytes of interest for clinical measurements are not usually the same ones that are of interest for biomonitoring studies. Therefore, the non-routine uses of blood or urine containers require an additional level of quality control.

Laboratory-performed quality control of lots of containers to be used for specimen acquisition will be required for the majority of methods. The screening procedure must assure that when specimens are collected and stored following laboratory protocol, the contamination introduced by the containers themselves or any preservatives is negligible (i.e. below the detection limit of the analytical method).

Pre-cleaning (acid-washing, solvent rinsing) of collection materials may be indicated for some analyses.

Collection blanks and multiple specimens can be collected alongside regular samples. A collection blank is a blank or empty specimen container from the same lot as the specimen containers used to collect participant specimens. In the absence of a prescreened container, use of a collection blank to estimate the extent of contamination introduced in the field may be necessary. It is of particular importance that collection blanks, to the extent possible, be subject to all the steps and manipulations to which study specimens are subjected. Multiple specimens may be collected for archival and future analyses. In studies, the inclusion of a field blank may be appropriate. A field blank is a blank or empty container the laboratory transfers to the sampling site for the purpose of determining ambient contamination levels in the field and in the laboratory. Additionally, a procedural blank may also be appropriate. Procedural blanks and field blanks are quite similar, but with the procedural blanks you start "tracking" from the time the samples are processed (e.g., make aliquots to separate them into specimens for analysis) instead of the actual collection. These field (procedural) blanks could be DI water on a urine collection cup and then processed as if it was an unknown sample (e.g., separate aliquots in cryovials, store, ship). For serum, DI water or a mixture of organic solvent (e.g., methanol) and water could be used.

Specimen collection procedure

Proper collection of specimens to be tested for environmental chemicals serves an important first step in assuring that the final results of laboratory testing are representative of concentrations actually present. Use of specimen collection instructions, field blanks and duplicate samples is recommended to standardize the collection procedure and estimate potential bias that may begin at the specimen collection stage.

Clinical and Laboratory Standards Institute (CLSI) publishes consensus standards covering the optimal procedures for the collection, handling and processing of commonly collected clinical specimens, such as venous blood and spot urine samples[23]. The CLSI standards, however, do not take into account some challenges unique to biomonitoring studies. First, since biomonitoring studies aim to measure environmental chemicals in humans, contamination from external sources at the time of collection must be minimized by careful specimen collection and strict adherence to study collection protocols. This is especially important in non-clinical settings such as private homes or community centers where biomonitoring specimens may be collected for convenience purposes.

Specimen collection instructions detailing collection procedures for specimens for use in a particular study are essential. The instructions should be written, with input from laboratorians and from healthcare providers (phlebotomists, nurses or physicians) involved in the collection. Step-by-step directions should be specific on where the sample containers are obtained, how they are labeled, what information should be collected prior to the collection, what steps need to be taken to prevent contamination, and where the collected specimen is to be routed for storage or transportation.

Instructions geared towards study participants should be written simply and without jargon and should include a phone number where clarifications can be obtained. In some situations, it may be advisable for laboratory staff to train those collecting and transporting specimens to minimize the likelihood of exogenous contamination and to ensure the viability of specimens during transport.

Specimen identification and documentation

Proper specimen identification remains paramount in any laboratory setting. The unambiguous identification of specimens is necessary to allow for the linking of laboratory results to demographic, dietary and/or lifestyle information also collected for the purpose of the study. Unless the purpose of the study is purely range-finding (i.e., designed to determine the range of concentrations for a particular chemical in a given population), the validity of the study conclusions will depend of the integrity of this link.

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[23]CLSI H01-A6 Tubes and Additives for Venous and Capillary Blood Specimen Collection; Approved Standard-Sixth Edition

GP16-A3 Urinalysis; Approved Guideline - Third Edition

GP34-A Validation and Verification of Tubes for Venous and Capillary Blood Specimen Collection; Approved Guideline

Unambiguous identification of specimens in biomonitoring studies using de-identified specimens can be complicated by the fact that patient names may not be listed on the specimen vials. Regardless of whether or not patient name is provided, the specimens should be labeled with at least two identifiers (i.e., study identification number and date of birth or other identifier). Bar coding of specimen vials and laboratory submission forms is recommended for the initial identification and efficient tracking of lab specimens in the laboratory utilizing a Laboratory Information Management System (LIMS). Additionally, it is important to note, the labels themselves should be known to remain sticky despite temperature and humidity changes that may occur during handling or storage.

As is the case with most laboratory test requests, standardized forms (whether hard copy or electronic) and protocols are generally used to make sure that all required information is collected and transmitted with the collected sample. This will include, at a minimum, study subject name (or ID code), a second identifier such as date of birth, date collected, specimen type and volume or test order. Subject's demographic information (gender, age, race, ethnicity) may also be included on the "test requisition form," or they may be collected elsewhere (e.g., while completing questionnaires regarding dietary habits or occupational exposure).

Shipping to the laboratory

Packaging and shipping of hazardous materials is highly regulated in the United States and internationally. Following hazardous material regulations is the responsibility of the shipper. However, for many biomonitoring applications, the laboratory will supply sampling kits and packaging materials. These supplies and their use for a particular mode of transport must be in compliance with regulations listed at the end of this section. In addition, laboratory personnel may have to refer some tests to other facilities and, therefore, must be in compliance with all applicable shipping regulations, including training of personnel required by these facilities. Laboratories that maintain contracted courier services should also be aware of packaging requirements for this type of transport.

The list below includes links to regulations pertaining to the transport of hazardous materials:

- Department of Transportation (DOT) regulates transport of hazardous materials by all modes of transport except for the U.S. Mail. Specific regulations (published in the Code of Federal Regulations, CFR, Title 49; parts 100-185) and relevant publications can be found at http://hazmat.dot.gov.
- International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO). ICAO regulates the international transport of dangerous goods or hazardous materials by air. The frequently-referenced IATA document "Dangerous Goods Regulations" resides at this website along with specific packaging instructions for infectious substances: http://www.iata.org/whatwedo/cargo/dangerous_goods/index.htm
- U. S. Postal Service or U.S. Mail Regulations affecting the transport of hazardous materials in the US mail, including Division 6.2 materials, are codified in the Code of Federal Register, 38 CFR and published in the Domestic Mail Manual: http://pe.usps.gov/text/dmm300/601.htm#1_0

Specimen storage and banking

Laboratory protocols for storage and handling of specimens should be part of the overall study protocol documents. Unless specimens are being stored for future use, procedures for when and how specimens will be destroyed at the end of the study are needed.

Once received at the laboratory, specimens must be stored properly to avoid target analyte and matrix deterioration. While room temperature storage may be appropriate for some matrices (e.g., hair or nail clippings), refrigerated or frozen storage is commonly employed for the majority of common clinical matrices such as urine, whole blood, serum, etc. Storage at temperature below freezing (-20˚C or -70˚C) is generally recommended for long-term storage and for temperature-sensitive analytes such as those to be speciated by oxidation state.

While these storage guidelines are widely followed, it should be noted that there is limited information on the stability of many analytes that may be chosen for biomonitoring studies. The effect of temperature variations during transport, prolonged storage or thawing on the concentration of the majority of analytes has not been studied. These effects remain particularly concerning when plans include specimen banking (i.e., long-term storage for future studies). Analyte stability studies would have to be undertaken to assess suitability of a chosen analyte and storage method for such applications. This information may be available through analytes studied through CDC's NHANES.

Analytical Testing

Analytical Protocol Template

The analytical protocol should contain the following information that details the laboratory steps and instrumental settings used.

Standard operating procedures (SOPs) serve as essential laboratory documents detailing a complete technique for analytical performance. An SOP is important to ensure operations are carried out correctly, consistently and in a reproducible manner. When used properly, SOPs can improve and maintain quality analytical methods, standardize laboratory performance and assure quality results. More information on SOPs can be found the CLSI document *Laboratory Documents: Development and Control; Approved Guideline – Fifth Edition*[24] Clinical Relevance and Summary of Test Principle

- Safety Precautions for Biological Hazards and Chemical Hazards
- Procedures for Collecting, Identifying, Storing, and Processing Specimens; Criteria for Specimen Rejection
- Preparation of Reagents, Calibrators (Standards), Controls, and All Other Materials; Equipment and Instrumentation
- Calibration, Calibration Curve and Calibration-Verification Procedures
- Operating Procedures; Calculations; Interpretation of Results
- Reportable Range of Results
- Quality Control (QC) Procedures
- Remedial Action if Calibration or Quality Control Systems Fail to Meet Acceptable Criteria
- Limitations of Method; Interfering Substances and Conditions
- Reference Ranges (Normal Values)
- Critical-Call Results (“Panic Values”)
- Specimen Storage and Handling During Testing
- Alternate Methods for Performing Test and Storing Specimens if Test System Fails
- Test-Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)
- Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking
- References

[24]http://www.clsi.org/source/orders/free/gp2-a5.pdf

Analytical Standards

Reliable chemical standards used to prepare calibration and control solutions remain essential to any analytical method. For biomonitoring applications, analytical standards will likely represent a significant cost of the total analysis.

Isotope dilution methodology is generally considered a reference method for accurate chemical measurements. The amount of biological material and interference encountered in clinical specimens cause a high level of "matrix effects" that can be overcome to a certain extent with the use of the isotopically-labeled internal standards. Accordingly, optimum analytical methods for biomonitoring frequently use isotopically-labeled compounds as internal standards. These compounds typically cost significantly more than the unlabeled compounds, but they provide a higher degree of precision and accuracy that cannot be obtained without them. Isotopically labeled standards are the ideal standards to use; however, they may not be available in all instances.

If the analytical method has been published and is being used routinely, commercial sources of analytical standards usually exist. Analytical standards may be purchased as solutions or as neat material. If the laboratory has the appropriate balance capabilities, it might be preferable to purchase neat material, which can then be made into appropriate solutions by the lab. This provides the lab with more flexibility in solvent selection and stock solution production. Care should be taken to be sure that purchased materials are of highest purity and receipt and expiration date are written on the standard material container.

In some instances analytical standards needed for a novel biomonitoring method may not be commercially available and may need to be synthesized.

Instrument Calibration

To establish a calibration curve, dilute stock standard solutions of appropriate concentration to prepare a series of calibration standards with the lowest concentration point on the calibration curve below the reported detection limit and the highest point above the expected range of results. The remaining calibration points are distributed between these two extremes with the majority of points in the range where most unknown sample concentrations are expected to fall.

For many biomonitoring methods, the calibration curve can be produced in a matrix (e.g. blank urine or blood) by spiking calibration solutions into appropriate matrix material and preparing them alongside the samples. This matrix-matching procedure is particularly necessary when there is a derivatization step or there is an enhancement or suppression that cannot be reproduced without matrix.

All calibration and control materials must be stored appropriately when not in use. This may include specific storage temperature and for some compounds, storage in the dark.

Quality Control

It is critical for laboratories to have proper bench (daily) quality control (QC) materials for their analytical systems (e.g. instruments) when analyzing samples, particularly for biomonitoring. Otherwise, the analytical processes or instruments could potentially show an erroneous increase or decrease in concentrations due to long term drift of the instrument or the analytical process. QC materials must be well characterized and stable for years, so that long-term QC can be tracked. Even a small analytical drift can result in an erroneous public health conclusion or decision because the difference over time for a group of people (group mean level) can be small, but it may be statistically significant. Quality control and proper laboratory techniques prevent erroneous conclusions, ensuring that the biomonitoring study results are valid and are scientifically defendable.

Quality control samples comprise part of each analytical run and demonstrate the accuracy of the method during each sample run. Quality control samples are typically the same matrix as the study specimens and have concentrations of target compounds in the low, medium and/or high range of the assay calibration.

If available, certified reference materials (CRM) or Standard Reference Materials (SRM) should be used to prepare QC samples. Unfortunately, CRM does not exist for many biomonitoring analytes of interest, and when it does exist, it tends to be expensive. If a commercially-available source cannot be located for the chemicals of interest, quality control materials can be prepared in the laboratory using blank matrix (see section below).

Blank QC material can be fortified with the appropriate level of the chemicals of interest. Optimally, the solutions used to fortify the QC material should be from a different source than the solutions used to generate calibration standards. If a second commercial source cannot be found, it would be best to produce a second set of stock solutions from neat material.

Due to the possibility of endogenous species, the QC material should be characterized after it has been fortified. QC characterization is accomplished with a minimum of 20 analytical runs to produce an average target concentration and a standard deviation from which to derive the limits. The laboratory will have to establish the system by which the QC results are evaluated and accepted or rejected. A good starting point for this system would the Westgard rules (http://www.westgard.com/westgard-rules/).

External verification of internally-prepared quality control materials is strongly encouraged. Public health laboratories may be willing to provide this verification testing. A listing of laboratories can be found on the APHL homepage[25].

[25]http://www.aphl.org/AboutAPHL/memberlabs/Pages/default.aspx

Blank matrix for calibration and/or quality control samples

Preparing the blank matrix serves the first, and perhaps, most important step in preparing calibration and quality control standards.

Blank urine can be frequently prepared in the laboratory. IRB approval may be required for urine collection from volunteers, but it is typically easy to obtain since the urine is pooled and de-identified. When this is done, typically one liter of urine per QC level will produce enough material to make it worth the effort. If the calibration standards are to be prepared in matrix, typically three liters of "blank" urine is needed.

If the matrix is blood/serum/plasma, large amounts of material can be obtained from local blood centers using the expired material that cannot be used on patients. Bovine blood may be also obtained commercially, but both bovine and blood center materials will likely need to be further characterized as free of the analytes of interest.

For methods designed for human breast milk, satisfactory matrix material can be found with infant formula. Typically, the soy-based products will contain less potential endogenous material than the bovine-based products.

For other matrices, the laboratory may be able to substitute one of the above materials depending on the situation. If possible, methods requiring material other than urine (due to the large volume of material required) or if blank urine can't be procured should use solvent-based calibration standards instead of matrix-based standards.

Method validation (internal and external)

Method validation remains necessary to confirm that the analytic method is suitable to detect, identify and reliably measure the target compounds in the designated matrix. Method validation is a set of experiments which demonstrate the accuracy, precision, selectivity, and sensitivity of the method. These experiments typically verify specific performance characteristics in order to produce a publication for the method or when a laboratory is adapting a method from another laboratory. The term "method verification" frequently describes the latter. See Appendix 6 for a Clinical Method Validation template.

Accuracy and precision of each method varies depending on the matrix, the analytical technique, and the analytical standards. Sufficient experiments on matrix-based samples allow determination of intra- and inter-day precision and accuracy. This may also be accomplished with the QC characterization using the 20 analytical runs minimum (see "Blank QC" above).

Typically, mass spectrometry and isotopically-labeled standards significantly improve a method's accuracy and precision; however, as long as the data generated are qualified, good methods can be produced without these techniques. As general guidance, method accuracy within 20% of the theoretical value would be considered sufficient for most applications. Precision at a given concentration level should not exceed 15% of the coefficient of variation[26]. Both the accuracy and precision become worse as the concentrations approach the limit of detection (LOD).

Selectivity of the method establishes that the correct component in a chromatogram is being measured as the chemical of interest. Selectivity is established using (1) the retention time of the chemical versus the analytical standard, (2) the specific mass being monitored in mass spectrometry, and (3) the ion abundance ratio of the chemical by monitoring at least two ions per chemical. If mass spectrometry is not used, or if a satisfactory second ion cannot be established, the retention time of the chemical on two different chromatographic phases can establish selectivity. Determination of selectivity should be done with control material and actual field samples to properly determine if all interferences have been identified.

Sensitivity of the method is the determination of the limit of detection (LOD). There are many ways to determine a method's LOD. A good reference for the determination of LODs for biomonitoring methods is described in *Quality Assurance of Chemical Measurements*[27]. When the method LOD has been determined, it is advisable for the calibration range to include standards at and below this LOD. Typically, only results within the calibration range can be reported by the laboratory, by establishing the calibration range below the LOD, the majority of identified results can then be reported.

Reportable range is also a component of method validation and is generally determined by the analysis of field samples. The calibration range should attempt to include the concentrations expected to be found in an unexposed population as well as the concentrations found in exposed populations. Many times, this range will be too great for the analytical technique and it may be necessary to analyze a diluted sample when a high result is found.

Stability of the chemical in and out of matrix will need to be determined, as samples can be collected over a long period and high-priced analytical standards will be used as long as possible. There are several types of stability that need to be addressed: freeze-thaw stability, short-term stability at room temperature, long-term stability in storage conditions and stability of the chemical in solvent as opposed to matrix.

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[26]http://www.ncbi.nlm.nih.gov/pmc/articles/PMC130103/

[27]J.K. Taylor, Quality Assurance of Chemical Measurements. Lewis Publishers, Chelsea, Michigan. 1987. pp. 78-84

With the analytical chemistry industry constantly improving techniques, the laboratory needs to have a mechanism to update and improve their methods. If significant changes are made to a method, it may be necessary to run a new set of validation experiments to determine the new accuracy, precision and sensitivity. If the new technique does not significantly impact these parameters, (e.g. updating a method to use a 96-well plate format as opposed to a vacuum box format) the modified method can be compared to the older method by split-sample analysis. With this technique, a minimum of 40 samples, and preferably 50 samples, are analyzed by each method. The results for the two analyses are then compared using a correlation plot with regression analysis and a difference plot with regression analysis. ICLN developed guidelines for Comparison of Validation Levels between Networks that may be helpful for biomonitoring studies[28].

Proficiency testing

Enrollment in an external proficiency testing program is the preferred way of confirming the accuracy of laboratory measurement as well as pre-and post-analytical laboratory procedures.

External: Proficiency testing enables a laboratory to establish its accuracy relative to other laboratories measuring the same chemical. Semi-annual testing is sufficient to establish the accuracy of the lab and the entire analytical system. There are many proficiency testing programs for inorganic analysis including a program run by the Wisconsin State Health Department, State of New York through the Wadsworth Center and CAP. For organic analysis, there are fewer programs including the AMAP program in Canada, and the German External Quality Assessment Scheme. Since the field of biomonitoring is not regulated and ever-expanding, proficiency testing programs do not typically exist for all the possible chemicals. When a program does not exist, it is advisable for the laboratory to seek out other laboratories doing the same measurement, in order to exchange quality control materials and compare the results. If enough labs are doing the measurement, a round-robin style of testing can be established where each lab measures the same QC material and the results are compared. The APHL Biomonitoring Database is a good resource to find laboratories to participate in a round-robin[29].

Internal: Within the laboratory, a proficiency testing program for analysts can be established by characterizing some QC material at several concentration levels. Many aliquots of the QC material are then produced and “blinded” by having one person, not associated with the analysis, randomize the vials and assign their own identification to them. Semi-annually, this person presents vials for analysis to each analyst and then compares the results to the characterized results. This technique mitigates the possibility of an analyst knowingly or unknowingly altering results because the theoretical value is known.

[28]https://www.icln.org/docs/sop.pdf
[29]http://www.aphl.org/aphlprograms/eh/biomonitoringdatabase/

Emergency Sample Analysis

The above discussion applies to routine analysis methods, utilized to characterize population exposure to environmentally-relevant concentrations of chemicals. At times, a laboratory may be called upon to analyze emergency samples for chemicals that may not have a validated method. Methods for emergency sample analysis should be very basic given the primary focus on the turn-around time. Typically, these are samples for a highly-exposed person or population and will not need the sensitivity required in routine biomonitoring methods. The number of samples may be much smaller than a typical biomonitoring project which allows the analyst to produce extracts that have more matrix since the long-term stability of the instrument is not an issue. These methods are typically focused on qualitative identification of the chemical and a semi-quantification of the result. After the emergency has passed, the lab can perform a more comprehensive quantitation if necessary.

SECTION V: RESULTS REPORTING

Generating, interpreting and communicating results remain critical components of any biomonitoring effort. This section addresses each of these three steps separately.

Generating Laboratory Result Reports

Ideally, the source of data for generating laboratory results is the laboratory information management system (LIMS). Typically laboratory data is collected on computer-controlled analytical instruments and this data is then transported to the LIMS. Data elements used by the analytical instrument must be mapped to the appropriate data elements in the LIMS.

For laboratories certified under the Clinical Laboratory Improvement Act of 1988 (CLIA), individual laboratory reports must contain certain mandatory elements:

- name, address and telephone number of the laboratory;
- two unique sample identifiers (typically these are name and date of birth but could also be any combination that includes those or the following: study identification number, medical record number);
- specimen type or source (urine, blood, serum etc);
- date of specimen collection;
- date of sample receipt;
- date of sample analysis;
- tests performed;
- results;
- reference ranges;
- any additional testing used to normalize contaminant levels, including name of the reference laboratory as appropriate;
- the date report was printed.

A full description of the CLIA requirements for laboratory reporting is available here: http://wwwn.cdc.gov/clia/regs/toc.aspx.

In addition to the required data, there are several desirable elements that should be considered for inclusion on laboratory reports if space permits:

- analytical methodology;
- detection limits;
- the CLIA certification number; and
- the name of the laboratory director.

See Appendix 4 for recommendations on ways to present chemical analyte levels of non-persistent chemicals in urine, persistent lipophilic chemicals in serum, etc.

Provision of Quality Control Data for Data Validation and Data Verification

Laboratories reporting confidential medical information electronically must ensure and validate periodically that electronic transmissions are consistent with hardcopy results produced and adhere to all relevant data privacy regulations and policies.

Results Interpretation

Determination of reference ranges, critical values and action levels is an essential step in interpreting and communicating the results of biomonitoring studies. Additionally, interpretation of biomonitoring results is improved when it is possible to compare results to a reference range. Therefore, the availability of reference ranges should be a consideration in the study design, selection of the biomarker and sample media. The reference population should be comparable to, and representative of, the study population.

Reference ranges indicate the concentrations of analytes expected to be found in the general population. Reference ranges may not always be available for all analytes of interest. The Centers for Disease Control and Prevention's *National Exposure Report*[30] serves as an excellent reference for selected compounds and elements. Alternatively, *Toxicological Profiles* prepared by the Agency for Toxic Substances and Disease Registry or the primary literature may have data regarding specific human levels. In the absence of established reference ranges, the laboratory must indicate that there are none on the laboratory reports. Occasionally, the laboratory will also report concentrations found in all study individuals as an aggregate report so that participants can see where they fall within the study population.

Critical values are those which may indicate higher than average levels. They typically trigger additional activities such as priority review of survey data to identify either potential exposure sources or confounders. Actions may include confirmatory or reflex testing by the laboratory.

Action levels are those which greatly exceed the expected clinical concentration warranting immediate notification of findings by the laboratory, so that not only additional sampling, testing and review be initiated, but also medical treatment begun if available.

[30] http://www.cdc.gov/exposurereport/

Results Communication

Results communication may involve returning individual data to specific participants or it may include the reporting of aggregate data more broadly. Factors to consider include ethical issues, how to respond to results that may be worrisome, the need for confirmatory or repeat sampling, and the possibility of legal or economic ramifications. Although the language and details will need to be tailored to each individual audience, the overall message should be consistent across all groups.

Plans for the communication of results should be determined in the initial study design, and also addressed as part of the informed consent process. Timelines for communicating results should be identified in advance. If community partners are involved, a communication plan should be jointly developed with them. The cost of regular communication with the community must be factored into the study plan.

Reporting Individual Results

Biomonitoring study participants should be provided their individual test results as soon as practical at the conclusion of the investigation. This requires careful coordination with the community, medical providers and public health officials to ensure that there are clinical support and risk communication teams assembled to provide appropriate answers that may result from the release of information.

Typically, the dissemination of individual results and aggregated data are synchronized so that individuals have not only their specific levels, but understand them within the context of their community. The notable exception to this procedure is for participants with significantly elevated results (at identified action levels) who should be notified during the course of the investigation to obtain additional information that may be relevant to the exposure, to collect a confirmatory sample or to initiate suitable medical treatment.

Public health agencies involved in biomonitoring should employ or contract with medical toxicologists, occupational health physicians and/or relevant medical specialists (i.e., pediatricians) to assist in crafting the message to participants. They may also directly advise participants (and their medical providers) regarding the significance of their individual results and any appropriate exposure reduction actions or medical interventions they might suggest.

Laboratories typically report results to the health department or medical provider rather than the individual study participant. CLIA regulations include a detailed listing of what is required.

Reporting Aggregate Results

Some studies will report aggregate data in addition to or instead of individual participant results. Although the focus in presenting aggregate results is not the individual, highly exposed individual(s) or groups can be identified by comparing individual results to (1) the overall study population, (2) comparable groups or (3) particular occupational groups.

The key elements of an aggregate report of results include:

1) Study purpose/objectives/hypothesis
2) Study design, including selection of biomarkers/biomatrices
3) Study sample
    a. Study population
    b. Sampling method
    c. Sample size/power estimation
4) Data quality
    a. Definition of variables
    b. Laboratory methods used to measure chemicals and their standardization
5) Statistical analysis
    a. Data Preparation-missing or miscoded data
    b. Description of data (n, mean, or median, sd or IQR, min, max)
    c. Inferential analysis
    d. Caveats in data analysis: how to deal with limit(s) of detection (LOD), missing ata, small sample size and outliers
6) Results
    a. Sample description
    b. Restatement of study objective(s)
    c. Results of statistical analysis with the statistical methods used
7) Interpretation/Communication

Various reports may be necessary depending upon the intended use and audience. For example, a detailed report documenting all methods, protocols, analyses and findings may be needed for collaborating agencies whereas an executive summary may be more appropriate for the public and policy makers. Consider the audience and setting when preparing the presentation of findings.

Reports should include the following elements:

Describe study purpose: List objectives, methods and procedures used. If applicable, state the study hypotheses. This will introduce justification for the study design.

Describe population studied: If the sample was intended to be representative of the target population of interest, comparisons can be made to show whether the sample is truly representative of the target population for defined variables of interest (e.g., age, sex, geographic location, ethnicity, etc.).

Present findings: Results for each research question should be presented individually in the same order proposed in the study objectives. For each research question, present the data, using tables and/or graphs, as appropriate. Information on the type of statistical test(s) performed and results of such tests, such as p-values, should be included.

a. Summarize highlights of findings by using tables showing the analytes to which people are exposed and at what concentrations.
b. Report the prevalence of people with levels above known specified toxicity levels, (e.g., a blood lead level greater than or equal to 10 micrograms per deciliter).
c. Report whether exposure levels are higher among certain groups, especially potentially vulnerable groups such as the elderly, pregnant women and children.
d. Report trends in levels of exposure of the population over time or geographical area if such data is available.

Interpret findings: Provide a narrative description of what the results mean with respect to a potential health risk. When possible, the results should be put in context by comparison with other appropriate biomonitoring data, e.g., other studies of comparable populations. If applicable, describe how these data differ from the general population as reported by CDC. Investigators will need to determine if the aggregate results will include an interpretation of "normal" vs. "high." If clinical reference values or health-based reference values are not available, determine whether other standards exist that are applicable (e.g., RfD, NIOSH RELS, other occupational standards). If health-based reference values are not available, state so, but try to provide comparisons with other available data. To the extent possible it will be important to provide an explanation whether observed differences in analyte levels by age, gender, or race/ethnicity are because of the differences in exposure, pharmacokinetics (absorption, distribution, metabolism, and excretion), or the relationship of dose per body weight.

Draw conclusions: Recommendations may be possible at two different levels. First, the findings may be useful in setting priorities both for public health efforts to reduce exposure to specific analytes and for research on human health effects. Second, they can be used to provide recommendations for reference or comparison values that can be used by physicians and scientists to determine whether a person or group has an unusually high exposure.

Communication with the Community

A communication plan should be part of the original study design and based on the combined principles of risk communication and health communication. The plan should outline communication goals and objectives, intended audiences, communication methods and products, timelines, and resources needed to effectively carry out the communication plan. As discussed above, the plan should specify whether individual participants will be able to receive their own results prior to releasing any aggregate data. If community partners are involved, a communication plan should be jointly developed with them.

Communicating with the public and policy makers requires thought and skill. Good communicators provide a complete picture of potential risks while avoiding the use of technical jargon. They also acknowledge the limitations of the available information and identify areas where additional data would be beneficial to understanding the risks and uncertainties. This is especially important in communities that are affected by a known source of chemical contamination. Ongoing communication efforts and integration of audience feedback are needed to ensure clarity of key messages and about the extent to which biomonitoring can inform and/or influence government remediation actions or legal actions against a responsible party.

In addition to the elements of the report outlined above, the report to the community should include additional explanation of the study methods including how study participants were chosen, and how data was collected, analyzed etc. The benefits and limitations of this approach should be clearly articulated.

To aid in the understanding of the interpretation of results, communicators should ensure clarity in explanations that the measurement of an environmental chemical in a person's body tissues or fluid provides an estimate of how much of a chemical is present in a person, but cannot necessarily predict what health effects, if any, may result from that exposure.

For some chemicals, it is important to explain that presence in the body alone does not indicate if the exposure was high or low, acute or chronic. Chemical levels in blood, serum, and urine are affected by how much of the chemical has entered the body through all routes of exposure, including ingestion, inhalation, and dermal absorption, and how the chemical is distributed in body tissues, transformed into metabolites, and eliminated from the body. Also, biomonitoring data alone do not pinpoint the exposure source, the route of exposure nor linkages between the chemical and adverse health effects.

For biomarkers that are not specific to a particular chemical, offer possible interpretation of findings. For chemicals that are also formed as a result of a normal metabolic process (such as formaldehyde and acetone), clearly explain that their presence cannot be attributed solely to an external exposure.

External Coordination

A variety of external agencies, organizations and entities will be important partners in the conduct of a biomonitoring study and in communication with the public and policy makers. The roles and responsibilities of these external stakeholders will vary depending on the level of the study. These stakeholders can also provide resources not available to the public health laboratory including access to data, target analyte information, patient samples and community groups. Laboratories should work with their environmental health program directors and state epidemiologists to establish relationships with external stakeholders and share information on their planned projects.

Local health: Local health departments serve as great partners and resources. They may be able to help laboratories and state epidemiologists identify local history and culture, reach communities of interest, provide essential communications support, or provide data.

State, local or federal environmental protection agencies: The US Environmental Protection Agency (EPA), Departments of Environmental Quality, Departments of Environmental Protection and other state or local environmental agencies remain excellent resources to gather data on environmental exposures, permissible limits (if applicable) and regulations regarding some chemicals.

Poison Control: Emergency rooms and hospitals already have a relationship with their local Poison Control Centers, who are also a potential resource for biomonitoring projects, particularly those related to emergency response. Poison Control Centers often track trends in poisonings and have yearly surveillance data. The American Association of Poison Control Centers[31] produces an annual report with statistics and information on poisonings in that calendar year. They are also a good source to finding your local Poison Control Center as well as information on some common chemicals and guidelines for patient management. Additionally, certified poison control centers have medical toxicologists who may be helpful as collaborators during the design, planning and interpreting results.

Local hospital and private medical providers: Such groups are important stakeholders for patient management, specimen collection, and identification of targeted study participants. Medical toxicologists in hospitals or private practices may be important partners in understanding the biomonitoring data (see also section 1).

[31] *http://www.aapcc.org/dnn/default.aspx*

NEPHTN: The National Environmental Public Health Tracking Network (Tracking Network) is a system of integrated health, exposure, and hazard information and data from a variety of national, state, and city sources. Currently, 23 state and local health departments have received funding from CDC to participate in the Tracking Network[32] and are collecting data on hazards in the environment. These state and local health departments are examining trends in the environment and health using surveillance techniques. While surveillance is an important tool in understanding exposures, it alone cannot usually determine whether something in the environment is causing a disease. States with a Tracking Program are great resources for laboratories starting or expanding biomonitoring projects and programs. For those laboratories not located in a Tracking state, they may consider reaching out to neighboring Tracking states, their local department of public health or a local university.

[32]http://ephtracking.cdc.gov/

CONCLUSION

Laboratory methodologies and instrumentation platforms are more technologically advanced than our understanding of the health effects of chemical exposure. Biomonitoring studies that are scientifically valid, comparable and robust are necessary to gain insight into the relationship between human exposures and diseases or other health conditions. This guidance aims to help standardize biomonitoring laboratory practice, performance and data across public health laboratories.

It is important to remember that biomonitoring is a collaborative effort and the laboratory methodologies and analyses are only one part of a broader effort. It is essential for chemists to be involved with epidemiologists and environmental health directors in the planning process of a study as early as possible to assist in the identification of appropriate biomarker(s) of interest. At the same time, input from toxicologists and other medical professionals is necessary to understand and interpret laboratory results. Involving the community and other external stakeholders is also necessary to report results and educate the community members.

Building these relationships is not only necessary for study accuracy, it is also necessary to gain support for expanding state biomonitoring programs and corresponding laboratory capacity. It will also help better answer the long-standing questions about environmental health and responding to the concerns of the community. Support for the program is necessary to secure the proper resources to see the study through to completion.

APHL hopes this guidance provides laboratories with the technical information needed to expand state biomonitoring programs. If you have suggestions for this document or any aspect of the biomonitoring system, please contact us. APHL is committed to further developing a national network of public health laboratories able to provide accurate human exposure data that will inform public health decisions through biomonitoring. As a leader in biomonitoring efforts, APHL continues enacting the five-year plan for public health laboratories as well as collaborating with partners and federal agencies. Part of this collaboration includes a guidance document for epidemiologists developed by the Council of State and Territorial Epidemiologists (CSTE.) The CSTE guidance document is meant as a companion to this guidance. If you are interested in sharing information about your biomonitoring project or would like more information about CDC's National Biomonitoring Program, please contact APHL at EH@aphl.org. Visit APHL's website to learn more about the Database and join to input your information (http://www.aphl.org/aphlprograms/eh/biomonitoringdatabase/).

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APPENDIX 1: GLOSSARY A-Z

Absorption: Process of active or passive transport of a substance into an organism: in the case of a mammal, such as a human being, this is usually through the lungs, gastrointestinal tract, or skin.
Action values (see also critical values): Action values are those which greatly exceed the expected clinical concentration warranting immediate notification of findings by the laboratory, so that not only can additional sampling, testing, review be initiated, but also medical treatment begun, if available.
Acute exposure: Short-term (in relation to exposure or effect) single contact with a substance or repeated contact over a 24-hour period of time.
Adverse effect (or adverse health effect): A change in biologic function or structure that leads to dysfunction or disease.
Analyte: A substance, such as a chemical, measured by a laboratory method.
Analytical chemistry[33]: Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much of it exists.

Bioaccumulation: Progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake exceeds the organism's ability to remove the substance from the body.
Bioavailability: Extent to which a substance to which the body is exposed (by ingestion, inhalation, injection, or skin contact) reaches the systemic circulation, and the rate at which this occurs.
Bioconcentration: Process leading to a higher concentration of a substance in an organism than in the environmental media to which the organization is exposed.
Biomarker: 1. Indicator signaling an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility. As related to biomonitoring, a biomarker is the presence of any substance, or a change in any biological structure or process that can be measured as a result of exposure to a substance. Many biomonitoring studies focus on chemical substances or their metabolites as biomarkers. 2. Parameter that can be used to identify an effect in an individual organism and which can be used in extrapolation between species for risk assessment.
Biomonitoring: The assessment of human exposure to environmental chemicals by measuring the chemicals or their metabolites in human specimens such as blood or urine.
Biosafety engineering controls : Laboratory protective equipment that acts as the primary barrier to hazards in the lab and includes biosafety cabinets and chemical hoods.
Body burden/chemical body burden: The total amount of a substance in the body.

Case control study (see also study design): A study that compares exposures of people who have a disease or condition (cases) with people who do not have the disease or condition (controls).

[33]http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=1188&content_id=CTP_003375&use_sec=true&sec_url_var=region1&__uuid=162e56f1-8756-42ab-9b22-a8ad13e4caef

Chronic exposure/long-term exposure: Contact with a substance that occurs over a long time (usually months to years).
Clean room: A confined area in which the humidity, temperature, particulate matter, and contamination are precisely controlled within specified parameters. The class of the clean room defines the maximum number of particles of 0.5-micrometer size or larger that may exist in one cubic foot of air in the designated area . For example, a class 1 clean room allows one such particle of any kind to exist in one cubic foot of space; a class 10 area may contain no more than 10 such particles in one cubic foot of space. (http://www.chemicool.com/definition/cleanroom.html)
CLIA: Clinical Laboratory Improvement Amendments
CMS: Centers for Medicare and Medicaid Services
Cohort study (see also study design): Looks at multiple health effects of an exposure; subjects are defined according to their exposure levels and followed for disease occurrence.
Collection blank: An empty specimen container from the same lot as the specimen containers used to collect participant specimens.
Compound: Substances composed of two or more stable chemicals.
Contaminant: A substance that is either present in an environment where it does not belong or is present at levels that might cause harmful (adverse) health effects.
Convenience sample: Participants are selected at the convenience of the scientist, not randomly.
Critical values (see also action values): Values which may indicate higher than average exposure and typically trigger additional activities such as priority review of survey data to identify potential exposure sources or confounders as well as confirmatory or reflex testing by the laboratory.
Cross-sectional study design (see also study design): Looks at relationship between exposure and disease prevalence in a defined population at a single point in time.
CRM (Certified reference material): Controls or standards used to check quality.

Ecologic study (see also study design): Looks at the relationship between exposure and outcome at population-level.
Epidemiology: Study of the distribution and the determinants of health-related states or events in populations as well as the application of the results to control health problems.
Exposure (see also acute and chronic exposure): Contact with a substance by swallowing, breathing, or touching the skin or eyes.
Exposure assessment: The process of finding out how people come into contact with a hazardous substance, how often and for how long they are in contact with the substance, and how much of the substance they are in contact with.

Field blank: An empty container (or a container filled with high-purity solvent) the laboratory transfers to the sampling site for the purpose of determining ambient contamination levels both in the field and in the laboratory.

GC-MS/MS (see also mass spectrometry): Gas chromatography tandem mass spectrometry; a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample.

Half-life: The time it takes for 50% of the original amount of a substance to disappear.

ICP-MS (see also mass spectrometry): Inductively-Coupled Plasma Mass Spectrometry; a method that combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.
IRB: Institutional Review Board
Isotopes: Atoms that contain the same number of protons but a different number of neutrons

Laboratory Information Management Systems (LIMS): A class of software that receives, processes, and stores information generated by laboratory processes and often interacts with laboratory instrumentation.
LC-MS/MS (see also mass spectrometry): Liquid chromatography tandem mass spectrometry; an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry.
Limit of Detection (LOD): The level at which the measurement has a 95% probability of being greater than zero.
Longitudinal study (see also study design): A correlational research study that involves repeated observations of the same items over long periods of time -- often many decades; a study that evaluates changes over time.

Mass spectrometry[34]: Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds, and to elucidate the structure and chemical properties of molecules. Detection of compounds can be accomplished with very minute quantities (as little as 10^{-12} g). This means that compounds can be identified at very low concentrations (one part in 10^{12}) in chemically complex mixtures.
Matrix: Specific sample types such as blood, urine or hair which in analytical chemistry are tested for the presence or absence of a compound or mixture (analyte).
Metabolism: The conversion or breakdown of a substance from one form to another by a living organism.
Metabolites: Any intermediate or product resulting from metabolism.

NHANES: National Health and Nutrition Examination Survey; ongoing survey designed to assess the health and nutritional status of adults and children in the United States.

Parent compound: A chemical compound that is the basis for one or more derivatives.
Persistence: Length of time a chemical remains in the environment or the body.

[34]http://www.asms.org/whatisms/p1.html

Pharmacokinetics : The study of what the body does to a drug (absorption, distribution, metabolism and excretion).
Prospective study (see also study design): A study in which the subjects are identified and then followed forward in time.

Randomized sample: Group of items or individuals from a larger population selected in such a way that all individuals from the population have an equal chance of being selected.
Reference values: Value (or range of values) that serves as a comparator, often used as to describe what is common or normal in a population.
Risk: The probability that something will cause injury or harm.

Samples/Specimens: Human (clinical) sample such as blood, urine, other bodily fluid or tissue taken for biomonitoring testing.
Serum: The liquid portion of blood that remains after the removal of clotting proteins and blood cells.
Statistics: A branch of mathematics that deals with collecting, reviewing, summarizing, and interpreting data or information. Statistics are used to determine whether differences between study groups are meaningful.
Study design: Broadly describes public health investigations involving human biomonitoring for surveillance, emergency response and research purposes.

Toxicant: Toxic or poisonous substance.
Toxicity: The degree to which a substance or mixture can harm humans or animals.
Toxicology: The study of the harmful effects of substances on humans or animals

UPS System: An uninterrupted power supply system is used for emergency power in the event of a power outage. This ensures computers and instrumentation do not loose information or data.

Glossary References

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APPENDIX 2: Statistical Analysis

Data preparation is the process of cleaning and organizing data for analysis. Data usually are gathered from many different sources, such as questionnaires, medical records, laboratory results, etc.

Checking the data analysis results against the primary sources or original forms used for data collection is often a necessary step. In order to be able to successfully track back records, there should be protocols established before data collection is initiated that address checking for data completeness and accuracy, for recording and keeping track of data, data entry, etc.

A data element dictionary should be created. The data element dictionary should include at minimum, the variable name, description, formats, codes, null value acceptance, access privileges, collection method, location in the database for each variable.

For data that will be manually entered into a database, such as hard copy questionnaires, measures should be defined to identify entry errors. It is also good idea to develop a log for documenting all QA/QC activities—recording who, when, how and why for any updates – so that changes can be understood or undone.

Descriptive analysis is used to describe the basic features of the data in a study. This type of analysis may be sufficient by itself when the aim is to provide a reference range. When defining a reference range, consider sampled population and factors affecting pharmacokinetics of specific chemicals, such as age, body mass index, genetics, disease, medication, alcohol, and diet.

Descriptive analysis should include findings related to each analyte measured in specific biomatrix (e.g., blood, serum, and/or urine) by sample size (n), percentage or results that fall below the limit of detection (%<LOD), arithmetic mean, geometric means, and percentiles (e.g. 10th, 25th, 50th, 75th, and 90th), with associated 95th percentile confidence intervals.

Geometric means or medians (50th percentile) generally are better estimates of central tendency than arithmetic means, because biomonitoring data usually have a distribution with a long tail at the upper end of the distribution. However, it is not recommended to calculate geometric means if more than 40% of data is below the LOD[35,36]. Percentiles will provide information about the shape of the distribution. The 90th or 95th percentile can be helpful for determining whether levels are unusually high.

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[35]US EPA. Report on Environment--Reporting Data Below the Limit of Detection. Available at: http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewMeta&ch=46&lShowInd=0&subtop=341&lv=list.listByChapter&r=224030. Accessed on 9/8/2010.

[36]CDC. NHANES. About the National Health and Nutrition Examination Survey—Introduction. Available at: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm. Accessed on 9/13/2010

[37]Helsel D. Nondetects and data analysis: Statistics for censored environmental data. Hoboken, New Jersey: John Wiley & Sons, Inc., 2005.

For each chemical, results should be presented for the total population sampled, as well as stratified by age group, gender, and race/ethnicity. Other demographic variables such as education or income may also be of interest if available.

Temporal trends can be estimated by comparing data collected over defined time periods. The non-parametric Kendall test can be used for trend detection; it is less affected by outliers, and it does not require fulfilling assumptions required for linear regression.

Inferential analysis is used to make inferences from the sampled data to more general conditions, and to look at relationships between chemical (biomarker) levels and variables relevant to the sample characteristics. For representative or population-based samples, sample weights will likely need to be applied to adjust for unequal probability of selection and also non-representativeness. Given that statistical models vary in their inferential utility, statistical consultation is recommended to determine which statistical models should be applied to the data set. Biomonitoring data are usually not normally distributed, and the data may need to be transformed or nonparametric methods may need to be employed.

Statistical methods used for inferential analysis depend on data type for outcome and explanatory variables (predictors) as well as the study objective.

Depending on study design, strength of relationship between an exposure and outcome is quantified using cumulative incidence or incidence rates in a cohort study, and odds ratio in case-control study. Table 1 below summarizes appropriate statistical analysis methods by data type.

Table 1. Guide for Statistical Analysis Method*

Objective	Data type		
	Continuous with normal distribution	Rank/score	Binomial/Categorical
Compare two groups	T-test	Mann-Whitney rank test	Chi-square or Fisher's exact test
Compare three or more groups	ANOVA (Analysis of Variance)	Kruskall-Wallis	Chi-square test
Describe direction and strength of relationship between two variables	Pearson correlation if the distribution are normal	Spearman correlation to quantify relationship if distribution are not normal	Contingency coefficients
Model outcome using predictors	Simple or multiple linear regression		Simple/Multiple logistic regression or factorial logistic regression or Discriminant analysis

*Biomonitoring data is usually not normally distributed. First check the data for normality and transform the data if necessary before applying parametric tests based on normality assumption.

Conclusion Validity

Whenever a study concludes that there is a relationship, conclusion validity should be discussed. Conclusion validity is whether a relationship is a reasonable one or not, given the data. The two possible error scenarios are: 1) to conclude that there is a relationship when in fact, there is not one (this is called Type I error or false positive or alpha (a) error); and 2) to conclude that there is no relationship when in fact, there is one (this is called Type II error or false negative or beta ß error).

In order to improve conclusion validity, researchers can choose a high statistical power, such as 0.9 or higher. This means the chances of finding a relationship when there is one (true positive) will be at least 90 chances out of 100 or more. One strategy to increase power is having a large sample size. Additionally, researchers can increase reliability by having good quality control and assurance measures as discussed above in the data quality section as well as in the sampling method section.

Caveats in statistical analysis of data

Instrumentation and analytical science improvements have made comparison of studies with significant time between the studies difficult. Four common problems faced with biomonitoring health data are analytical limits of detection (LOD), missing data, small sample size and outliers.

Limit of detection (LOD) is the level at which the measurement has a 95% probability of being greater than zero[38]. As laboratory methods evolve, LOD values change over time. The LODs for each analyte and the proportion of samples that fall below the LOD (%<LOD) should be provided in each data table and collectively in an appendix. Methods used to assign a value to analytical results <LOD in data analysis should be described and referenced. If LOD values change during the study period, the most conservative approach is to use the highest LOD value[39].

For most chemicals, the LOD is constant for each individual specimen analyzed. For dioxins, furans, PCBs, organochlorine pesticides, and a few other pesticides, each individual sample has its own LOD. These analyses have an individual LOD for each sample, mostly because the sample volume used for analysis differs for each sample. A higher sample volume results in a better ability to detect low levels, and a lower LOD. It is not uncommon to get results below the LOD especially when the exposure to a certain chemical is low. Various statistical methods have been developed to address this issue[40,41,42].Therefore it is important to partner with a statistician to determine how best to interpret results and to resolve issues related to LOD.

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[38]John Keenan Taylor. Quality assurance of chemical measurements—Principals of Measurement.CRC Press LLC. Boca Raton, FL. P:79

[39]CDC. NHANES. Key Concepts about the Limit of Detection (LOD) of Environmental Chemicals. Available at: http://www.cdc.gov/nchs/tutorials/environmental/critical_issues/limitations/Info2.htm. accessed on 9/8/2010.

[40]Finkelstein MM, Verma DK. Exposure estimation in the presence of nondetectable values: another look. AIHAJ 2001;62:195–198.

Missing data: The three common reasons for missing data are true missing data, refusal to answer, and "don't know." Methods for dealing with missing data must be clearly defined. First, determine if there is a pattern for missing data and if it is necessary to make adjustments to avoid non-response bias. The lower the response rate, the higher the non-response bias possibility.

Small sample size might lead to unreliable data. Combining several years of data based on sample size and power calculations might help dealing. A minimum sample size of 30 is recommended for reporting any descriptive statistics[43].

Outliers: Methods are needed for defining, identifying, and dealing with data outliers in the data analysis. It is important to review all values defined as outliers to make sure coding errors were not made. Data can be analyzed both with and without the outlying cases to see how results differ. Justification is necessary for including or excluding outliers – including why the outlier does not really fall into the population of interest or why the outlier values differ so much from the rest. Transforming data, using square roots and logarithms, softens the impact of outliers. As a last resort, consider deleting outliers, but note how doing so changes the summary statistics.

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[41]Lubin JH, Colt JS, Camann D, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect 2004;112:1691–1696.
[42]Helsel D. Nondetects and data analysis: Statistics for censored environmental data. Hoboken, New Jersey: John Wiley & Sons, Inc., 2005.
[43]National Center for Health Statistics, Centers for Disease Control and Prevention. Analytic and Reporting Guidelines. The National Health and Nutrition Examination Survey (NHANES), September, 2006 Hyattsville, Maryland
[44]2003 Requirements Document Publication -16 PHLs, the Association of Public Health Laboratories (APHL), and the Public Health Informatics Institute, the Centers for Disease Control and Prevention (CDC), Public Health Information Network (PHIN)

APPENDIX 3: Summary of Informatics Issues Relevant to Biomonitoring

Once thought of as a support function, the delivery of laboratory IT services has now evolved to the point where electronic recordkeeping and automated data management are mission-critical components of public laboratory operations. And while laboratories may once have had complete control over essential informatics activities, more often than not, this is not the case today.

The Laboratory Information Management Systems (LIMS) and the infrastructure that supports the LIMS are among the most important technologies in a public health laboratory. LIMS are typically directly connected to analytical instrumentation. The interfacing of these analytical devices to the LIMS has become an integral part of the analytical process. LIMS implementation has become highly collaborative through efforts such as APHL and new federal data-sharing requirements which include a comprehensive set of 500 or more LIMS requirements that span across 16 specific business processes[44].

The LIMS themselves are highly specialized IT installations tailored to the kinds of laboratory work being performed. The long-term success of LIMS implementations requires PHL leaders to thoroughly plan and appropriately budget for the design, acquisition, installation, and maintenance phases of the LIMS project cycle.

The LIMS familiar to virtually all governmental laboratory directors is only the most visible component of the laboratory's IT infrastructure; the proverbial 'tip of the iceberg.' To be sure, technologies such as the LIMS and associated hardware and software are critical assets. However, the larger IT infrastructure also includes:

- **Governance functions,** such as contract oversight, budgeting for IT products and services, policymaking and other management activities.
- **Technical support,** including software customization, staff training, trouble-shooting and other activities to implement commercial technologies and otherwise assist end-users.

The management of IT may lie outside of the laboratory and IT services may be shared or consolidated within a larger organization structure. Like state laboratories, shared IT services arrangements can take many forms however there are some common approaches that laboratory leaders can use to negotiate with IT leaders. A recommendation is to first focus on the totality of the laboratory IT infrastructure (which is more than just the laboratory information management system). Additionally, memoranda of understanding (MOUs) and service level agreements (SLAs) are the two major tools recommended for IT services negotiations and ongoing management; these document the IT activities that are necessary for successful laboratory operations. IT and laboratory leaders can use these tools to communicate and document the costs, risks and metrics of laboratory IT services. The documents must convey the importance and functions of laboratory services, and be written in the language of the IT professional with clear business case models.

Table 1 summarizes support activities that the MOU or SLA should encompass. Table 2 summarizes many of the informatics components that are necessary to support a laboratory LIMS and automated data handling.

1. Summary of Multiple IT Support Functions Necessary for Successful Laboratory Operation

- Ability to meet complex customer data requirements of multiple state and federal public health agencies.
- Ability to meet rapid response times associated with emergency response and surge capacity, requires scalability and high availability 24/7.
- Ability to store and retrieve large amounts of analytical data; fully redundant and configured for no data loss to ensure continuity of operations.
- Ability to maintain high levels of security for infectious and toxic agencies tracked by the LIMS- Laboratory personnel with access to this data must maintain security clearance such as FBI secret security level clearance.
- Ability to standardize laboratory data collection and reporting of measurement quality objectives to assure interoperability with other national laboratory partners.
- Ability to better manage laboratory fiscal and business needs.
- Ability to manage the increased complexity associated with laboratory deliverables including complex reporting and security requirements such as CDC's select agent rule, CLIA, HIPAA, MITA, etc.
- Ability to integrate complex analytical instrumentation and automation into data collection and reporting.
- Ability to integrate data and interoperability connect with other laboratories and federal agencies.
- Ability to serve within a national implementation as much as an individual state implementation, with PHLs acting as a group.
- Ability to provide the necessary bandwidth for data communication.

2. Summary of Multiple IT Services Necessary for Successful Laboratory Operation

Operational Services	Backups, schedule job and performance monitoring, and other support functions
Service/Help desk	It is necessary to have systems and processes to efficiently and completely handle 10's to 100's of support, service and project requests daily.
IT Training	To cover topics from basic network and desktop software use, security, regulatory requirements, data messaging etc.
Development Services	To build custom reports, implement components of the LIMS, and support other operational systems
Other	Security enhancement tools, legacy application modernization, records management

While there are many perceived differences in laboratories, on closer examination and exploration – the laboratories are organized differently but had many informatics commonalities. These commonalities lead to the ability to collaborate and share common data sets. Both nationally and internationally, public health networks depend on the ability of LIMS to share data interoperably[45].

Interoperability can be looked at as an approach to extend data collection and exchange beyond the individual laboratory and jurisdiction. As laboratories automate many current services, the future for laboratory informatics may include implementing solutions that are multi-directional that promote the goals of nation-wide laboratory data exchange.

APHL's PHLIP (Public Health Lab Interoperability Project) is a successful interoperability model to consider for multi-laboratory biomonitoring collaborations. The goal of PHLIP is collaboration. PHLIP's vision is for improved data quality and accessibility with increased distribution of pertinent health data for faster decision making for the patient and the greater community. For more information on interoperability visit the Informatics Program at APHL's website, www.aphl.org.

[45]The term "Interoperable" describes the technical requirements for bringing two systems together to work in concert with each other to serve a common purpose. Interoperability allows for discreet informatics systems to be unique in what they do and how they deal with data but, when they exchange data it is understood on a similar context.

Electronic Messaging of Analytical Results

The Electronic Data Deliverable

In this age of increased electronic communication, it is common for data users to request laboratory data in a standardized electronic format also known as an Electronic Data Deliverable (EDD). Reporting EDDs saves laboratorians time by sending data directly from a Laboratory Information Management System (LIMS), minimizing and possibly eliminating manual data entry. Additionally, EDDs reduce transcription errors and speed up data delivery in a secure manner. For the data user, EDDs save time by standardizing the data collected from multiple laboratories using multiple analyses. It also allows the use of automated data review software to approve and share data. Overall, EDDs minimize the need to harmonize and cleanse data.

Laboratories reporting confidential medical information electronically must ensure and validate periodically that electronic transmissions are consistent with hardcopy results produced and adhere to all relevant data privacy regulations and policies.

Given the extreme diversity in laboratory information management systems and the various formats and reporting requirements of response agencies, the creation of a standard to address analytical reporting of environmental health and environmental results is critical. Two references are recommended. APHL has a policy position paper "An Interoperable and Integrated Federal Data Exchange Network for Environmental and Environmental Health Data[46]." Also, there is a white paper which serves as a reference document for standardized electronic data exchange. "Environmental Laboratory Electronic Data Management" prepared by APHL's Environmental Health Committee, Environmental Laboratory Subcommittee, and Informatics Committee[47]. APHL has a draft EDD, based on EPA's Environmental Response Laboratory Network data deliverable. It is matrix-independent, method- independent and program-independent in an effort to increase standardization across programs. Please contact EH@aphl.org for a copy.

[46]http://www.aphl.org/policy/Documents/2010/Policy_2010March_DataExchangePositionStatement.pdf

[47]http://www.aphl.org/aphlprograms/eh/Documents/EDMWhitePaperFinal.pdf.

Reporting the Electronic Data Deliverable

EDDs have different formats depending upon the data consumer. Sometimes the results can be provided as a spreadsheet, where every column represents a data element such as sample number, specimen type or source (urine, blood, serum etc), date of specimen collection, date of sample receipt, tests performed, results, result units, etc. Results can also be provided in languages intended for machine readability such as eXtensible markup language (XML) or Health Level 7 (HL7).

An XML file is a structured file that contains data. It is a type of database. It uses author-created tags to surround and organize content, like an outline. The design goals of XML emphasize simplicity, generality, and usability over the Internet [6]. It is a textual data format with strong support via Unicode for the languages of the world. Although the design of XML focuses on documents, it is widely used for the representation of arbitrary data structures, for example in web services. XML allows data elements to be related to each other. These relationships facilitate data review and interpretation.

Typically clinical data is reported using HL7, which is an all-volunteer, non-profit organization involved in development of international healthcare informatics interoperability standards. “HL7” is also used to refer to some of the specific standards created by the organization (e.g., HL7 v2.x, v3.0, HL7 RIM). CDC has a version of HL7 tailored for biomonitoring and clinical chemical data exchange that can exchange associated quality control data along with the specimen results.

HL7 and its members provide a framework (and related standards) for the exchange, integration, sharing, and retrieval of electronic HL7 v2.x of the standards, which support clinical practice and the management, delivery, and evaluation of health services, are the most commonly used.

Including Quality Control Data within the Electronic Data Deliverable

Increasingly, data users are requesting electronic data deliverables (EDDs) that include the raw measurement quality control data and which can meet unique measurement quality objectives (MQOs). APHL supports this trend: sharing quality control data, adds to certification or accreditation by providing specific accountability to each result data result set.

As an example, in addition to the results of field generated specimens, the quality control data may include laboratory-generated (positive and negative control) samples, target and non-target substances, some batching information, instrument performance and calibration information. To provide this quality control data, each quality control sample is reported just like patient specimens, each with a unique specimen laboratory number and all result associated data elements like analytic name, results, analysis time, method, units, etc.

Policies, Brokering, and Nomenclature

Policies, brokering, and nomenclature details are critical technical issues for electronic data exchange. Policies are necessary to address security issues. Record content and messaging protocols are necessary to provide significant security constraints on EDDs. Data standards that include nomenclature, content, analyte valid values etc. need to be understood before mapping between multiple formats if possible.

Any LIMS implementation must address valid values for each data element. These constraints on the value sets define the allowable values for an EDD. For older laboratories with a legacy of method names and allowable values, these valid values may require complex translators to migrate data. Newer implementations between agencies may resolve brokerage by allowing a direct LIMS-to-LIMS data exchange where the valid values are part of the interface. More typically, data is exchanged from separate systems and requires an intermediate stage using translators to broker data exchange. These translators can be on the data generator or the data consuming end: internal facing or external facing.

Automated Data Reporting Tools

Lastly, there is a desire for data review software that can serve as a data checker to assure that data meets formatting and nomenclature requirements. Data review software is also useful for data users that seek to rapidly review reported results against client requests and method quality objectives. By providing this information, data generators and consumers can review a data submission and know that all the data measurements submitted match what the results generated.

Secure Transport of Electronic Data Deliverables

EDDs can be transported in different ways. Two issues need to be addressed: 1) the transport mechanism and 2) the security of the data. Sharing of patient information is regulated under HIPAA and requires strict security measures.

The most basic approach (and least recommended) to transport data is to attach a spreadsheet EDD to an email and send the email. This option is considered easy for the data generator to send and the data consumer to receive, but this approach limits program data review and automated usage and provides limited data security.

Clinical data transport in the realm of health care is highly secure and employs machine readable messages. The current approach for data transport of public health infectious disease information is to place the data into an HL7 message. To use a metaphor: HL7 is the letter, and PHIN MS is the postal carrier (requires envelopes to look just so in order to deliver them). PHIN MS is one such messaging option. Others include NHIN Direct, Active encryption, Certificates (state and other authorities), VPN, SSL, sFTP.

APPENDIX 4: Recommendation for Ways to Present Chemical Analyte Levels:

1. Non-persistent chemicals measured in urine
   a) per volume of urine
   b) per gram of creatinine (adjusted for urinary creatinine)

   * Levels per gram of creatinine (i.e., creatinine corrected) adjust for urine dilution or concentration in spot urine samples. However, creatinine correction can partially adjust for differences in lean body mass or renal function[48].

2. Per specific gravity Measurement is less affected by age, gender, body size and meat in take than is creatinine adjustment. Specific gravity adjustment seems especially appropriate when comparing individuals or populations with large differences in muscle mass or meat intake[49].

3. Persistent lipophilic chemicals measured in serum
   a) Lipophilic chemicals(such as dioxins, PCBs, PBDEs, and organochlorine -pesticides, are presented
      i. weight of chemical per kilogram of total lipid (microgram/kg of total lipid)
         or
      ii. per whole weight of serum. Serum levels reported per gram of total lipid reflect the amount of these compounds that are stored in body fat.
   b) Non-lipophilic chemicals measured in serum: expressed per liter of serum (e.g., microgram per liter).

4. Chemicals which are bound to hemoglobin such as acrylamide and glycidamide, which are bound to hemoglobin, are usually presented as picomoles per gram of blood hemoglobin.

5. Chemicals measured in whole blood, such as metals, measured in whole blood, are presented as weight of chemical per volume of whole blood (micrograms per liter).

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[48]Garde AH, Hansen AM, Kristiansen J, Knudsen LE. Comparison of uncertainties related to standardization of urine samples with volume and creatinine concentration.Ann Occup Hyg. 2004 Mar;48(2):171-9.

[49]Moriguchi J, Ezaki T, Tsukahara T, Furuki K, Fukui Y, Okamoto S, Ukai H, Sakurai H, Shimbo S, Ikeda M. Comparative evaluation of four urinary tubular dysfunction markers, with special references to the effects of aging and correction for creatinine concentration. Toxicol Lett. 2003 Aug 28;143(3):279-90.

APPENDIX 5: Chain of Custody

Maintaining and processing Chain of Custody is an important aspect to any study involving human samples. This appendix includes a comprehensive example based on one laboratory's process and documents.

Initiating and Maintaining a Chain of Custody (COC) Document

Once a sample has been determined to require a COC, the Sample Collector must initiate the COC. The COC is initiated and maintained by all who transport and/or receive the sample within an organization/hospital. This legal document helps to ensure that the integrity of the sample is preserved. Do not transport chain-of-custody forms with specimens. Once the specimens leave the facility, the chain-of-custody stays behind. Each entity or organization handling the specimens is responsible for the specimens only during the time that it has control of the specimens. Each entity or organization receiving the specimens must sign-off on the chain-of-custody form of the entity or organization relinquishing the specimens to close that chain. Electronic procedures such as electronic chain-of-custody and barcode readers will expedite this process. When receiving specimens, each new entity or organization must begin its own chain of custody. The entity or organization relinquishing the specimens must sign its chain of custody to close the chain and indicate that they have transferred the specimens.

Note: When the person relinquishing the specimens (relinquisher) and the person receiving the specimens (receiver) are not together at the time of specimen transfer, the relinquisher must document on its chain-of-custody form that the receiver is the express courier (e.g., FedEx, Delta Dash, DHL, UPS) and must document the shipment tracking number or have the person transporting the specimens sign the chain-of-custody to indicate that he or she has taken control of the specimens. Likewise, when receivers get the specimens, they will document on their chain-of-custody form that the relinquisher is the express courier (and provide the tracking number) or have the person transporting the specimens sign the chain-of-custody form.
*The Sample Collector may either use his/her organization's own COC or the one provided by your laboratory. All fields must be filled in completely with ink.

Instructions:

1. Ensure that the Clinical specimen ID numbers (or a range of ID numbers for multiple specimens) is provided in the designated space.
2. The Sample Collector first prints and then signs their name.
3. The "Date" and "Time" must reflect the actual time of collection.
4. The "Organization" line must include the FULL name of the organization/hospital (no acronyms).
5. Include the full mailing address and telephone number of the organization/hospital.
6. Any person subsequently receiving or transporting the specimen must fill out the next "Received by" section of the COC.

7. Continue these steps for all subsequent Sample Couriers/Operators or Sample Custodians until the sample leaves the organization.
8. Once sample has left the organization, keep the COC internally for your records.

Note: The "Date" and "Time" must reflect the PRECISE time and date at which custody was transferred from the previous person to the new person. Because this information relieves the previous person from custody, it is essential that the new Sample Custodian notes this date and time as precisely as possible. Also, include the new Sample Custodian's name (printed), signature, and telephone number in the spaces provided Under "Organization," include without acronyms the organization represented by the new Sample Custodian.

In general, a sample requiring a COC will follow a path as follows:

Sample Collector -> Sample Courier/Operator -> Sample Custodian

However, it is important to note that anyone who receives or transports a suspect Select Agent must complete the appropriate section(s) of the COC.

Acronyms and Definitions

Chain of Custody (COC) – a written legal document used to track the transfer of a sample(s) from person to person.

Sample Collector - for clinical samples sent from hospitals, this would be the person forwarding the sample to the CTRL.

Sample Courier/Operator – the person responsible for transporting the sample from the Sample Collector to the CTRL.

Sample Custodian – the person who receives the sample (e.g., CTRL personnel), and has demonstrated competency in handling of samples and maintaining a COC.

Agency Name
Laboratory Name
City, State ZIP

External Chain of Custudy

INSTRUCTIONS: This form must be completed for any specimen that might be used in enforcement proceedings or litigation.

Transportation: During transportation of the specimen from collection site to the laboratory, the chain of custody must be unbroken. If the integrity of the specimen is questionable, describe the problem on the reverse side of this form.

Identifying #	Collection Date	Specimen Type	Number of Specimen	Comment
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	
		O Blood O Urine	# #	

CUSTODY OF SPECIMENS

	NAME	Affiliation	Date	Time
1. Specimens Collected by	_____	_____	__/__/__	_____
2. Specimens Shipped by	_____	_____	__/__/__	_____
3. Specimens Received by	_____	_____	__/__/__	_____
4. Specimens Received by	_____	_____	__/__/__	_____
5. Specimens Received by	_____	_____	__/__/__	_____
6. Specimens Received by	_____	_____	__/__/__	_____
7. Specimens Received by	_____	_____	__/__/__	_____
8. Specimens Received by	_____	_____	__/__/__	_____
9. Specimens Received by	_____	_____	__/__/__	_____
10. Received [Insert Laboratory Name] by	_____	_____	__/__/__	_____

APPENDIX 6: Clinical Method Validation Example

Validation Plan for Analyte detection In Human Urine by Liquid Chromatography/Inductively Coupled Plasma-Mass Spectrometry (LC/ICP-MS)

Introduction

This plan outlines the validation for measuring various analytes in urine by Liquid Chromatography/ Inductively Coupled Plasma Mass Spectrometry (LC/ICP-MS). This is simply provided an example; all biomonitoring work should follow all the data quality objectives practiced by the respective public health laboratory or sponsoring organization.

1. **Accuracy**
    a. Two controls (high and low) for each analyte will be run 20 times each.
    b. The percent recovery for each test value will be calculated.
    c. % recovery = [test value/actual value*100]
    * The percent recovery must be between 80-120%

2. **Precision (intra- and inter-run variability)**
    a. Two concentrations (high and low) of each analyte will be run.
    b. Twenty replicates of each concentration will be run over a minimum of two runs on different days.
    c. The following calculations will be performed:
        i. Mean
        ii. Standard deviation
        iii. % CV
        * The within-run % CV must be < 10%.
        * The % CV for samples run on both days must be < 15%.

3. **Specificity**
    a. Run individual species standards (made from neat standards) to verify retention times of each species.

4. **Range Validation**

    Linearity: correlation coefficient (R^2)
    a. A standard curve consisting of five levels (1 ppb, 5 ppb, 10 ppb, 50 ppb, 100 ppb) will be run for each analyte to assess linearity of the standard curve.
    b. Each level of the standard curve will be run in triplicate.
    c. The mean of the three values will be determined.
    d. The correlation coefficient of the standard curve using the mean values will be determined.
    * The correlation coefficient must > 0.990.

Analytical Sensitivity[50] (the smallest amount of an analyte in the sample that can accurately be measured by the method)

a. Minimum detectable limit (MDL) may be determined according to 40 CFR 136, EPA MDL Procedure.
b. For each analyte, a blank urine sample will be spiked to equal the concentration the lowest calibration standard.
c. Seven replicates will be analyzed (n=7).
d. The standard deviation (SD) will be calculated.
e. Using the equation [MDL = (SD) X (3.143)*], MDL will be calculated.
f. *Student t value for n-1 Degrees of Freedom

5. **Reportable range** (the numeric range of analyte concentration over which the method is able to produce certifiable accurate values). Reliable quantitation limit (RQL) will be determined by multiplying the MDL by 4.
   a. Linearity beyond upper boundary of curve will be demonstrated.
      i. A spiked sample 2x the concentration of the highest calibration standard (200 ppb) will be run as part of the standard curve.
      ii. The curve must demonstrate linearity ($R^2 \geq 0.990$).
      iii. The sample will then be diluted, run again, and the concentration calculated.
      iv. The two values must be within +/- 20%.

6. **Reference Range**
   Varies by analyte/matrix combination and/or age or occupational sub-populations.

7. **Westgard Rules**
   a. The mean data will be plotted along with +/- 2SD and +/- 3SD.
   b. The data will automatically be evaluated by Westgard Rules, and any failing results will be flagged.
   c. If a run fails the Westgard rules, it will be rejected.
   d. When the data passes the Westgard rules, it will be accepted for validation statistics.

[50]Taylor, J.K. Quality assurance of chemical measurements—Principals of Measurement. CRC Press LLC. Boca Raton, FL. P:79

1 3S Rule	Run result is outside a 3Si limit	Random Error
2 2S Rule	Current and previous run results are outside the same 2Si limit	Systematic Error
10 X-bar Rule	Current and previous nine run results are on same side of the characterization mean	Systematic Error
R 4S Rule	The current and the previous run results differ by more than 4Si	Systematic Error

Laboratory Supervisor signature	Date
Laboratory Division Director signature	Date
QA Manager signature	Date
Laboratory Director	Date

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