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Control of Preanalytical Variation in Trace Element Determinations; Approved Guideline

This document provides guidelines for patient preparation, specimen collection, transport, and processing for the measurement of trace elements in a variety of biological matrices.

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Control of Preanalytical Variation in Trace Element Determinations; Approved Guideline

Abstract

Control of Preanalytical Variation in Trace Element Determinations; Approved Guideline (NCCLS document C38-A) is intended for persons responsible for the collection and processing of samples used for trace element determinations. The guideline addresses patient preparation, as well as considerations for collection, transport, and processing of specimens by element. Contamination control and quality assurance programs are also discussed.

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Contents

Foreword

Preanalytical factors are probably the most important cause of erroneous trace element reference data in biological matrices today. The development of sensitive, specific, and accurate analytical technology at an acceptable cost has moved determination of trace and ultratrace elements from research facilities into a wide range of clinical laboratories. Expanding knowledge of trace element nutrition and toxicity has increased clinical demand for these assays. However, with increased sensitivity and lower limits of detection, the problem of specimen contamination with the element of interest has been magnified. It is vital that the accurately determined trace element concentration reflects the condition of the patient and not contamination introduced during collection and handling. Elements are classified according to the level at which they occur in the body as "trace" (body content 0.01 to 100 μg/g; 10 to 10^4 μg/L) or "ultratrace" (body content less than 0.01 μg/g; less than 10 μg/L).

Earlier attempts to define reference interval data for many of the trace and ultratrace elements provided ranges that were far wider than are now accepted as "normal." This resulted from a lack of awareness that the ubiquity of many trace elements in the environment required special precautions from preanalytical processes through the actual analysis.

In this document, the components of specimen collection and preanalytical processing that can contribute to trace element contamination are addressed and protocols for prevention of contamination are described. The trace elements most commonly tested for clinical purposes are individually listed. For each element, the optimal specimen for assessment, preanalytical factors to consider in patient preparation and reference intervals, or concentrations suggesting toxicity or deficiency, are described.

Key Words

Trace element, ultratrace element, essential elements, specimen collection, contamination control.

Control of Preanalytical Variation in Trace Element Determinations; Approved Guideline

1 Introduction

It is recognized that much of the pioneering research published in trace element literature is based on erroneously derived reference interval data.[1] The source of the problem was in the lack of recognition of exogenous specimen contamination, which could have occurred at the collection, transport, processing, or analytical stages. Thus, reference intervals for ultratrace elements, such as chromium, or acceptable blood concentrations for toxic elements, such as aluminum, have decreased several fold over the past two decades.

The use of increasingly sensitive methods, such as electrothermal atomic absorption spectrometry (ETAAS) or inductively coupled plasma mass spectrometry (ICPMS); increasing interest in ultratrace elements; and the need for precise and accurate analyses for elements such as lead, at extremely low levels, have accentuated the problems of analytical and preanalytical contamination.[2]

The intent of this guideline is to (1) develop an awareness of the factors that affect the determination of trace elements in a variety of specimen types, (2) foster communication between the laboratorian performing the test and those responsible for collecting the specimen, and (3) provide definitive protocols for eliminating preanalytical variability.

If a specimen is to be sent to a reference trace element laboratory for analysis, it is suggested that the laboratory be consulted in advance for special collection and handling instructions.

2 Scope

This guideline provides directions for patient preparation, specimen collection, transport, and processing for analysis of trace elements in biological matrices (i.e., body fluids, such as blood, urine, breast milk, and tissues). Specific reference is made to those elements that are known to be essential or toxic for humans and are, therefore, most likely to be measured for clinical reasons.

2.1 Definitions

For the purposes of this document, the following definitions apply:

Trace element, *n* - An element that occurs at a level of 0.01 to 100 μg/g (10 μg/L to 10^4 μg/L).[1]

Ultratrace element, *n* - Arbitrarily defined as one that occurs at a level of less than 0.01 μg/g (less than 10 μg/L).[1]

From the perspective of preventing preanalytical or analytical contamination, classification of an element as trace or ultratrace depends on (1) the expected concentration in the sample matrix and (2) the sensitivity of the analytical method used for that element in a specific matrix. Thus, for example, while aluminum occurs in the serum of healthy persons as an ultratrace element, in a patient on dialysis who has aluminum toxicity, aluminum may be considered a trace element. Tables 1 and 2 categorize clinically important elements found in blood and urine.

Essential element, *n* - That a specific trace element is consistently detectable in human tissues or fluids does not imply that it is essential. Many trace elements are so ubiquitous in the environment (e.g., Al, Pb) that it is hardly surprising that they are "normally" found in human tissues and fluids. As analytical detection limits are improved further, other rare elements could also be detected at ultratrace levels. The criteria used to establish essentiality in other areas of life science, e.g., plant growth[3,4] can be adapted, with some qualification, to the animal kingdom. An element is considered essential (a) if without it, the species cannot achieve normal, healthy growth or complete its normal life cycle and (b) if it is part of a molecule of an essential constituent or metabolite. In addition, the element must be specific and not be replaceable by another, and it must exert its effect, directly on growth or metabolism

and not by some indirect effect, such as antagonism of another element present at toxic levels.

Based on these criteria, a number of trace elements have been clearly identified as essential for normal, healthy growth in humans. While there may be some elements that are not universally accepted, due to the paucity of data supporting claims for essentiality, they may be considered borderline candidates. The concept of essentiality, and arguments over accepted criteria, are discussed in detail by Davies.[5]

Tables 1 and 2 list those trace and ultra trace elements, which are the focus of this document, in alphabetical order. Some are considered essential for normal, healthy growth in humans, others are borderline. Several are nonessential toxic elements. Elements in Groups I, II, and VII (i.e. the alkali and alkaline earth metals, and the halogens) are not included, although some of these are essential.

2.2 Reporting Units

A variety of units are currently used throughout the United States for reporting trace element concentrations in human body fluids and tissues (see Table 3). Although NCCLS documents generally use units that are fully acceptable within the Système International d'Unités (SI), these do not always coincide with the units recommended by the International Union of Pure and Applied Chemistry (IUPAC) and by the International Federation of Clinical Chemistry (IFCC) for reporting results of clinical laboratory measurements. Because SI units are used worldwide but there is not yet a consensus in the United States, NCCLS documents include the IUPAC/IFCC recommended units of volume (L) and substance (molecular) concentration (mol/L) in parentheses, where appropriate. In this document, wherever possible, we use conventional mass/volume (e.g., μg/dL) units, or mass/mass (μg/g) units, to describe normal and abnormal concentration ranges, followed by IUPAC/IFCC-recommended equivalents in parentheses.

Results for trace elements in urine can be calculated as an excretion rate if a timed specimen is obtained. Usually, such results are reported as μg (or mg) element per 24 hours, or as μg element per g (urinary) creatinine (see Section 5.2.2). In the analytical laboratory, it is commonplace to use "bench" units, such as parts-per-million (ppm) or parts-per-billion (ppb) for concentration. *These units should not be used to report trace element concentrations in clinical specimens*. They are confusing and ambiguous to nonanalytical personnel, since they do not indicate if the concentration is based on a mass/volume or a mass/mass ratio.

Table 1. Categorization of Elements Measured in Blood

Element	Atomic Number	Atomic Weight	Essential (toxic)	Concentration in μg/L (nmol/L)	Comments and Precautions	Ultra-trace Protocol
Measured in serum or plasma						
Aluminum (Al)	13	26.98	nonessential (toxic)	<10 (<370)	Avoid fruits, juices, and tea for 24 hours (citric acid).	yes
Cobalt (Co)	27	58.93	essential	<0.3 (<5)	Avoid beer for 24 hours.	yes
Chromium (Cr)	24	52.00	essential Cr^{3+} (toxic Cr^{6+})	0.04–0.39 (0.769–7.50)	Avoid worksite collection.	yes
Copper (Cu)	29	63.55	essential	age dependent	Require age-, sex-, and gestation- specific reference values. Note oral contraceptive or estrogen use and acute phase reactant.	no
Iron (Fe)	26	55.85	essential	age dependent	Fasting morning specimen and avoid diurnal variation. Require age-, sex-, and gestation- specific reference values. Note oral contraceptive or estrogen use and acute phase reactant.	no
Manganese (Mn)	25	54.94	essential	0.5 -1.8 (9.1-32.8)	Avoid worksite collection.	yes
Molybdenum (Mo)	42	95.94	essential	0.5-3.0 (5.2-31.3)		yes
Nickel (Ni)	28	58.69	uncertain	<2 (<34)	Avoid worksite collection.	yes
Selenium (Se)	34	78.96	essential	age dependent geographic dependent	Require age-, sex-, and gestation- specific reference values. Reference ranges affected by location; local ranges needed.	no
Vanadium (V)	23	50.941	uncertain	<1.0 (<19.6)		
Zinc (Zn)	30	65.39	essential	age dependent	Fasting morning specimen, avoid diurnal variation. Require age-, sex-, and gestation- specific reference values. Affected by albumin concentration.	no
Measured in whole blood						
Cadmium (Cd)	48	112.41	nonessential (toxic)	0.3-1.2 (2.7-10.7)	Avoid worksite collection	yes
Lead (Pb)	82	207.2	nonessential (toxic)	<100 in children (<0.48 μmol/L)	Avoid worksite collection.	no
Mercury (Hg)	80	200.59	nonessential (toxic)	<5 (<25)	Avoid mercurial antiseptics. Avoid worksite collection.	yes

Table 2. Categorization of Elements Found in Urine

Element	Atomic Number	Atomic Weight	Essential (toxic)	Concentration μg/24 hours (nmol/d)	Comments
Arsenic (As)	33	74.922	uncertain (toxic)	50 (0.67 μmol/d)	Avoid worksite collection. Avoid seafood ingestion, which increases excretion.
Cadmium (Cd)	48	112.41	nonessential (toxic)	1 (8.9)	High levels in smokers. Avoid worksite collection.
Chromium (Cr)	24	51.996	essential	0.05–0.58 μg/L (0.962–11.15 mmol/L)	Avoid worksite collection.
Copper (Cu)	29	63.546	essential	<38 (<0.6 μmol/d)	Increased by penicillamine challenge.
Lead (Pb)	82	207.2	nonessential (toxic)	<0.06 μmol/24 hours	8-hour urine collection used for chelation challenge. Avoid worksite collection.
Mercury (Hg)	80	200.59	nonessential (toxic)	0.1-2 (0.5-10)	Best to assess inorganic mercury. Avoid worksite collection.
Uranium (U)	92	238.029	nonessential (toxic)	0.012-26 μg/L (0.05-109 nmol/L)	

Table 3. Recommended Reporting Units

Preferred Units	Acceptable Equivalents	Acceptable Alternatives	Unacceptable
Biological Fluids			
Micrograms-per-liter (μg/L) Micromoles†-per-liter (μmol/L; μmol)	Nanograms-per-milliliter (ng/mL)	Micrograms*-per-deciliter (μg/dL) Micrograms-per-100 milliliters (μg/100 mL)	Parts-per-billion (ppb) Microgram percent (μg %; mcg %)
Milligrams-per-liter (mg/L) Millimoles†-per-Liter (mmol/L; mmol)	Micrograms-per-milliliter (μg/mL)	Milligrams‡-per-deciliter (mg/dL) Milligrams-per-100 milliliters (mg/100 mL)	Parts-per-million (ppm) Milligram percent (mg %)
Biological Tissues Based on Dry Weight			
Micrograms-per-kilograms (μg/kg)	Nanograms-per-gram (ng/g)	Micromole-per-kilogram (μmol/kg; μM)	Parts-per-billion (ppb)
Micrograms-per-gram (μg/g)	Milligrams-per-kilogram (mg/kg) Nanograms-per-milligram (ng/mg)	Millimoles†-per-kilogram (mmol/kg; mM)	Parts-per-million (ppm)

* Multiply by 10 to convert to micrograms per liter (μg/L).
† Requires conversion factor based on the relative atomic mass.
‡ Multiply by 10 to convert to milligrams per liter (mg/L).

3 Universal Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with universal precautions. Guidelines for specimen handling are available from the U. S. Centers for Disease Control and Prevention [*MMWR* 1987;36 (suppl 2S):2S–18S]. NCCLS document M29— *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, deals specifically with this issue.

4 Contamination Control

4.1 Background

Sample contamination during the collection process and during the preparation for analysis in the laboratory is a major problem and can lead to substantial errors. No matter how sophisticated the analytical instrumentation used to measure trace element concentrations, if the sample collection and preparation steps are not designed to minimize contamination, the results can be meaningless.

Contamination errors are so common in trace element analysis that special precautions must be taken to eliminate or reduce them. The severity of the problem depends on the specific element being determined, its natural abundance, and on the concentration level(s) desired/expected in the sample. For example, the effects of contamination errors in the determination of lead in blood at a concentration of 10 μg/dL (0.48 μmol/L) are quite different from those encountered when measuring copper in serum at a concentration of 110 μg/dL (17.3 μmol/L). Similarly, the process of measuring aluminum in serum at 10 μg/L (370 nmol/L; 0.37 μmol/L) is far more prone to contamination errors compared to measuring selenium in serum at 100 μg/L (1.27 μmol/L) because aluminum is a more ubiquitous element.

Contamination can occur at any stage of the preanalytical process, including specimen collection, transport, specimen handling, and preparation (drying, homogenization, grinding, or sieving).

In accordance with NCCLS policy, commercial product names are not used in this guideline. However, *it is mandatory* for any laboratory performing trace element determinations *to ensure that* all collection devices, reagents, and consumables used in collection, transportation, storage, or analysis will not contribute to the contamination of the specimen by the trace element of interest.[2,6] No container or device, even if stated to be suitable for trace-element analysis, should be used until evaluated (see Section 4.4). Furthermore, once a particular brand is chosen, each new lot must be similarly checked because random contamination can occur.

4.2 Contamination from Collection Materials

4.2.1 Specimen Collection and Storage Containers

Sampling materials and storage containers for body fluids are a major potential source of contamination. It is recommended that each laboratory has a written procedure in place for checking that all blood collection devices and associated equipment for trace element determination are contamination free. Two approaches for evaluating contamination are acid leaching and sera leaching. Sample protocols are provided in Sections 4.4.1 and 4.4.2, respectively.

Needles, catheters, and other nonfillable equipment should be checked by leaching in a solution of 4% (v/v) ultrapure grade acetic acid or 2% (v/v) ultrapure grade nitric acid for about 12 hours. The leachate should be analyzed for the element of interest using the method of choice, i.e., a method that can detect the lower limit of the biologically expected range. Any detectable level will trigger further investigation because it will be important to establish if the level of contamination is clinically significant.

An alternative approach is to test a random sample of collection devices from a lot for contamination by filling them with blood/serum that has the element of interest in a known low concentration that is at, or near, the method-detection limit. Subsequent

analysis should reveal any clinically significant systematic contamination of that lot. This is often not feasible in a pediatric environment.

4.2.2 Anticoagulants

When a blood sample is required, the optimal specimen may be whole blood, plasma, or serum (see Section 5.0). If whole blood or plasma is selected, an anticoagulant is required. Heparin or Ethylenediaminetetraacetate (EDTA) may be used, but each has advantages and disadvantages. Nevertheless, it is mandatory to check the preferred anticoagulant with regard to its potential for contamination with the element of interest.

EDTA is the preferred anticoagulant when specimens are to be transported with more than 36 hours delay. However, as a good chelating ligand, EDTA is easily contaminated with metal ions during the manufacturing process. Heparin is less likely to be contaminated and can be obtained as essentially "trace element free." However, heparin is generally considered a less reliable anticoagulant for long duration (24 to 36 hours). Often the choice between EDTA and heparin is based on known problems with the analytical method. Some manufacturers of evacuated glass tubes certify them for trace element analyses. Each lot should be checked for specific contamination problems before proceeding with the analysis. The use of materials "off the shelf" without any contamination check is not recommended. (See Section 4.2.1.)

Collecting and transporting fingerstick blood for lead screening purposes can be easily accomplished using a variety of commercial microcollection devices available. Most are available with either heparin or EDTA anticoagulants, but samples of each lot should be checked for gross systematic contamination before being used routinely.

4.2.3 Acid-washing "Labware"

Generally, all laboratory glassware and plasticware should be acid-washed by soaking overnight in solution of 10% (v/v) nitric acid, and given a final wash with Type I water. (Please see the most current version of NCCLS document C3— *Preparation and Testing of Reagent Water in the Clinical Laboratory.*) In some applications, commercial cleaning solutions (containing, among other things EDTA, polyethoxyphenol, and various alkyl sulfonic acids) have proven effective in removing residual aluminum contamination. With respect to plasticware, it is generally agreed that colored plasticware should be avoided because it is likely to be contaminated with trace elements.

4.3 Contamination from the Laboratory Environment

4.3.1 Dust-Free Conditions

Although a Class-100 clean room is desirable for trace element analysis, it is not always practical to set up such a room in most clinical laboratory settings. A laminar flow hood should be used for ultratrace element work. For many elements, a laminar-flow hood might not be necessary, provided that certain precautions are taken to eliminate the most common sources of contamination. Laboratory bench areas should be "wet wiped" frequently and floors should be "wet mopped" frequently to control dust contamination.

When weighing out reagents and dry samples, the analytical balance should be located in a dust-free cabinet. All laboratory supplies (plasticware and glassware) should be acid-washed before use and dried, if necessary, under dust-free conditions. Dust-free cabinets can be constructed specifically for this purpose.

For handling biological fluids and wet tissues of human origin, where aerosol-generating procedures may be employed (e.g., pipetting), a Class II biohazard safety cabinet should be used.

4.3.2 Reagent-Grade Deionized Water

Use of high-grade deionized water is essential for quality trace element analysis. Generally, several mixed-bed ion exchange cartridges are employed in-line to remove metal ions, with a third containing activated carbon and a fourth

containing a resin to remove organic contaminants. Various parameters are used to characterize the quality of deionized water, but for trace element work the resistivity of the water is a reasonable guide to its quality. Typically, a resistivity of greater than 10 megohms per centimeter (MΩ/cm) at 25° C is considered Type I water by several organizations.[7,8]

4.3.3 Ultrapure Reagents

4.3.3.1 Chemical Reagents

Only reagents of high purity should be used for trace element analysis. Several sources of high-purity materials are available, including the major reagent manufacturers and other specialty houses. Each lot of materials should be checked for unacceptable levels of contamination before proceeding with the analysis. Depending on the element being determined, American Chemical Society (ACS) reagent grade can be satisfactory. Very often, only a single source of high-purity material can be found.

4.3.3.2 Acids

Ultrapure acids are critical to high-quality trace element analysis, and most acid manufacturers provide concentrated acids in an ultrapure state. For some special applications, sub-boiling point-distilled nitric acid stored in plastic can be required. Such material is available commercially.

4.4 Testing Contamination in Phlebotomy Tubes

Before blood is drawn into an evacuated tube or syringe, a representative sampling of each lot of tubes must be examined to determine the extent of trace element contamination from the tube and stopper. The phlebotomy needle or syringe needle can also be checked by this process.

4.4.1 Acid Leaching

(1) To evaluate a new tube or new lot of tubes, select at least five, preferably ten, tubes from several cartons of tubes.

(2) In a working environment, as described in Section 4.3, remove the stoppers/caps from the tubes. For syringes, remove the top and pull the plunger down to accept 5 mL of solution. Pipet 5 mL of an aqueous leaching solution containing 5 mL of concentrated reagent-grade nitric acid per 100 mL of Type I water into each tube or syringe. Acid-leached pipet tips should be used to dispense the leaching solution. Reserve some of the leach solution for a reagent blank.

(3) Insert the stoppers back into the tubes and invert the tubes several times. Allow the tubes to leach in a horizontal position so that the solution is in contact with the stoppers. The contact time can vary from several hours to overnight (12 hours).

(4) At the completion of step 3, remove the stoppers and prepare an aliquot of the leachate as a sample for the element of interest. Also, prepare the unused leaching solution.

(5) Analyze the aliquots to determine the extent of contamination in the tube or syringe. Subtract the residual trace element content of the leaching solution from the concentration of trace element determined in the tube leachate.

(6) Ideally, the concentration of ultratrace, trace, or toxic elements should be at, or less than, the detection limit of the analytical technique.

4.4.2 Sera Leaching

An alternate procedure involves the use of pooled serum for the test mentioned previously. If pooled sera are available for which reference values are known, the pool can be used to evaluate the selected tubes in the same manner as in Section 4.4.1.

4.4.3 Phlebotomy Needles

Syringe or phlebotomy needles can be evaluated in a manner that is similar to the procedure described in Section 4.4.1.

(1) Select at least five, preferably ten needles from several boxes of needles.

(2) Use a trace element-free syringe or trace element-free evacuated tubes to draw leaching solution through the needles. The leachates should remain in the trace element-free evacuated tube or syringe until testing. For a trace element-free evacuated tube, not only are the needles tested, but the potential contamination from puncturing the rubber stopper is evaluated with this procedure.

(3) Analyze the leachate as described in Section 4.4.1(5).

5 Specimen Selection and Collection Protocols

The laboratory should not permit specimens for trace element determinations to be sampled for other tests outside the trace element laboratory section until the trace element determination has been performed or an adequate sample has been processed for this purpose.

The following caveats apply to all collection protocols throughout this guideline, but they will not be reiterated at each stage.

- Use only collection, storage, or handling devices that have been validated for the element of interest by your own laboratory or the laboratory performing the analysis.
- Validate each new lot of materials to ensure that systematic contamination has not occurred (see Section 4.2.1).
- Ensure that rigorous protocols for collection and handling of the specimen are followed. Where collections occur on wards, in clinics, or in physicians' offices, ensure that the personnel responsible for collections are properly trained and understand the importance of adhering to the protocols.
- Use only *nonpowdered* gloves throughout the collection procedure.
- Never freeze blood samples in glass tubes because the glass can fracture, which can cause the tube contents to leak out.

Although collection procedures are summarized in the following sections, more detailed protocols can be found in the following NCCLS documents:

- H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture.*[9]
- H4—*Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture*.[10]
- GP16—*Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens.*[11]

NOTE: The procedures within this guideline are intended to prevent trace element contamination, not microbial contamination.

5.1 Blood, Plasma, or Serum

Any discussion of specimen collection must be based on the target trace element for which the specimen is to be taken. Careful consideration must first be given to the kind of blood sample required: whole blood, serum, or plasma. For lead, cadmium, and mercury, whole blood is the specimen of choice. For most others, serum or plasma is preferred.

Protocols for the collection of blood, plasma, or serum must be based on an appropriate selection of the type of specimen to be obtained. The magnitude of the potential for exogenous contamination and the relative significance compared to expected endogenous levels must be considered. Thus, more stringent collection protocols are required for serum collected for ultratrace elements, such as chromium and manganese, than for selenium.

The method of sample collection will also depend on the element being determined. For diagnostic tests, venous blood is the preferred sample because it is unlikely to be compromised by contamination. Blood, especially from young children, is more convenient to obtain using the fingerstick

method, but this method is more prone to contamination errors. One area where fingerstick blood sampling has proven reasonably successful is in mass screening programs for childhood lead poisoning. The small number of gross contamination errors is considered acceptable because they result in false-positive rather than false-negative results, and because parents find such a practice more acceptable than venipuncture. Nevertheless, scrupulous cleaning of the fingerstick site is required, and some recommend special cleaning procedures to minimize contamination errors (see Section 5.1.5 on the procedure for collecting skin puncture specimens).

5.1.1 Whole Blood

Use only an evacuated anticoagulated tube that has been specifically designated for trace element collection and that has been checked by your laboratory or your referral laboratory. Suitable tubes are also available for pediatric collections. The following recommendations apply:

- The wound site should be cleaned with alcohol rather than iodine-containing disinfectants.
- If a series of tubes are to be collected from the same site, contamination is minimalized if the initial specimen is used for other tests and blood for trace elements is collected last.
- Only stainless steel needles with no aluminum or other metal crimp ring should be used. The needle should protrude through the hub and be secured by epoxy.

After blood collection, the tube should be inverted several times to thoroughly mix the anticoagulant with the blood.

- *Do not* open the blood collection tube. Send or ship the tube directly to the laboratory.
- Trace element specimens must remain sealed until received by the laboratory.

5.1.2 Plasma

Use only an evacuated anticoagulated tube that has been designated specifically for trace element collection and that has been checked by your laboratory or your referral laboratory. Suitable tubes are also available for pediatric collections. General guidelines for the collection of whole blood from Section 5.1.1 apply here. Specifically, the first four recommendations in Section 5.1.1 should be followed. The following recommendations apply:

(1) Centrifuge the tube for 10 min at a relative centrifugal force (RCF) of 1,000– 1,200 x *g* to separate the plasma from the erythrocytes and leukocytes.

(2) Remove the stopper and *pour* plasma into an acid-leached, plastic, screw-topped tube. Glass tubes are not acceptable as storage tubes. *Do not* touch plasma with any utensils, unless they have been acid-washed. If transfer pipets are used for pediatric specimens, they must be trace element free or acid-washed.

(3) Send the plasma directly to the laboratory.

5.1.3 Serum

Standard red-topped evacuated tubes or plastic syringes with black rubber tipped plungers are grossly contaminated with zinc and other trace elements and are *not* acceptable for sample collection when trace elements are to be determined. Several evacuated trace element tubes are available. The suitability of a given product must always be evaluated for the specific element to be tested. General guidelines for the collection of whole blood from Section 5.1.1 apply here also. Specifically, the first four recommendations should be followed.

(1) Allow the blood to clot for at least 30 minutes at room temperature.

(2) Centrifuge the tube for ten minutes at an RCF of 1,000–1,200 x *g* to separate the serum from the clot.

(3) Remove the stopper and *pour* the serum into an acid-leached, plastic, screw-topped tube. Glass tubes are not acceptable as storage tubes. *Do not* ream the sample with a wooden stick. *Do not* touch the serum with any utensils, unless they have been acid-washed. If transfer pipets are used for pediatric specimens, they must be trace element free or acid-washed.

(4) Send the serum directly to the laboratory.

5.1.4 Special Collection Protocol for Ultratrace Elements (Al, Co, Cr, Mn, Mo)

This collection should not be undertaken without consultation with the referral trace element laboratory.

If analyses for ultratrace elements are to have any validity, absolutely meticulous attention must be paid to the selection of collection devices and anticoagulants and to the collection protocol. All syringes are to be acid-washed (see Section 4.2.3), rinsed with deionized water, dried in particle-free air, and sterilized before use.

For ultratrace elements drawn through a Teflon® tube, the initial draw should not be used for the element analysis.

Blood is collected using a Teflon® catheter into a special acid-washed, centrifugable syringe as follows:

(1) Remove the plastic needle cover from a Teflon® IV catheter and insert the catheter into a vein. Carefully withdraw the needle from within the Teflon catheter by grasping the plastic base.

(2) Attach a small plastic syringe to the catheter and withdraw 2- to 3-mL of blood. Detach this syringe and discard it or use it for other nontrace element chemistries. (This step removes any metal contamination remaining from the needle insertion.)

(3) Remove the cap from a sterile, acid-washed, 10-mL syringe. Attach the syringe to the catheter and withdraw 10 mL of blood. Remove the catheter from the vein and recap the syringe. Unscrew the plunger shaft from the syringe and discard the shaft.

(4) Transport the blood specimen in the syringe to the laboratory immediately.

(5) Allow the blood to clot at room temperature in the syringe for a minimum of 5 h (maximum 24 h). Do *not* refrigerate.

(6) Centrifuge the syringe for 10 min at a relative centrifugal force (RCF) of 1,000–1,200 x *g*.

(7) While in the laminar flow hood, unscrew the top of the syringe. Transfer the serum using an acid-washed, plastic pipet, into an acid-washed polypropylene tube.

NOTE: If syringes that are used for collection are not able to be centrifuged, the blood is transferred to an acid-washed polypropylene tube for clotting and centrifugation. A second polypropylene tube is used for serum transfer.

5.1.5 Special Considerations for Collection by Skin Puncture

5.1.5.1 Patient Preparation

(1) Wearing powder-free gloves, thoroughly wash the patient's hands with soap and water, then dry them using appropriate toweling.

(2) Using thumb and index finger, grasp the patient's finger that has been selected for puncture. The palm of the patient's hand should be facing up.

(3) Gently massage the fleshy portion of the finger.

(4) Clean the ball or pad of the finger to be punctured with an alcohol swab. Dry the fingertip using a sterile cotton ball or gauze pad.

5.1.5.2 Collection Technique

(1) Grasping the finger, quickly puncture the pad of the finger with a sterile lancet, in a position slightly lateral to the center of the finger.

(2) Discard the first drop of blood using a sterile cotton ball or gauze pad.

(3) If blood flow is inadequate, gently massage the proximal portion of the finger, then press firmly on the distal joint of the finger. Do not "milk" or repeatedly squeeze the finger.

(4) After a well-beaded drop of blood forms at the puncture site, turn the patient's hand over so that the blood drop flows toward the floor. Ensure that the blood does not run down the finger or around the fingernail area.

(5) Continuing to grasp the finger, touch the tip of the collection container to the beaded drop of blood. Blood will be drawn into the container; a continuous flow of blood should be maintained.

(6) When full, cap or seal the collection container as appropriate.

(7) Agitate the specimen to mix the anticoagulant thoroughly with the blood.

(8) Check that the container is properly labeled, and place it in an appropriate storage area.

(9) Stop the bleeding and cover the finger with an adhesive bandage.

(**NOTE:** For information on skin puncture collection devices, consult the most current version of NCCLS document H14— *Devices for Collection of Skin Puncture Blood Specimens*.)

5.2 Urine

Urine excretion may be used for the monitoring of heavy metal overload in following up some therapeutic mobilization tests, such as Desferal® (Fe, Al), EDTA (Pb), or D Penicillamine (Cu), and for the investigation of a deficiency mechanism.[6]

5.2.1 24-Hour Collection

If urinary excretion data is required, a 24-hour urine collection yields the preferred specimen. Results should also be corrected for creatinine.

One exception is for a mobilization test for lead poisoning where an 8-hour collection is recommended (see Section 5.2.2.1).

Urine collection should be arranged away from the suspected exposure site. If workplace exposure is suspected, the collection should be taken during off hours, preferably days without any occupational exposure, away from the work environment (to avoid contamination from work clothing or atmosphere). The procedure is as follows:

(1) The patient must be provided with an acid- leached, wide-mouthed voiding container or a funnel for urine collection and an acid-washed *plastic* collection container. Metal or porcelain collection containers must be avoided. *Do not* use metal urinals or pans. The collection container should be kept refrigerated during the 24-hour collection period.

(2) For some trace element determinations, urine must be stabilized by the addition of ultrapure nitric acid to the collection container to lower the pH to <2. Usually, 20 mL of 6 mol/L nitric acid is adequate. Mix thoroughly.

(3) Measure a 50–100-mL aliquot in a trace element-free measuring cylinder. Measure the remaining volume before discarding it to obtain 24-hour volume.

(4) Put the 50–100-mL urine aliquot from step 3 into acid-leached, plastic, screw-capped bottles. *Do not* use the plastic jars with metal lids typically found in hospital settings or urinalysis laboratories.

5.2.2 Random Urine

A 24-hour urine collection is preferable; however, a urine specimen collected in a random manner is sometimes used for screening (arsenic, mercury) in cases of occupational exposure. The simultaneous measurement of urinary creatinine and reporting as μg element/gram creatinine may assist with the validity of the result interpretation (WHO document, special publication #647, Geneva, 1980). For example, [(μg/L cadmium × 100) / (mg/dL creatinine)] = μg cadmium/ g creatinine. However, this is not always used.

A random urine void may be collected by using a suitable plastic container with a plastic lid. Typically, 4.5-oz (130-mL) sterile containers are available in a physician's office or in a hospital setting. Before these containers are used for collection, their suitability for the element of concern should be validated (see Section 4.2.1).

The entire urine spot collection can be delivered to the laboratory or a small portion (5 mL) can be poured off into a plastic screw-cap vial and sent to the laboratory. In this case, the sample need not be acidified and can be frozen.

5.2.2.1 Calcium Disodium EDTA Lead Mobilization Test

The 8-hour collection for the $CaNa_2EDTA$ provocation or mobilization test is designed to provoke a brisk lead diuresis in which the total amount of lead excreted over an 8-hour period is measured. The test is no longer recommended for routine cases, but it does remain a potentially useful tool in clinical research.

All materials used to collect urine and transport the specimen to the laboratory must be checked and certified as lead-free. Therefore, it is the responsibility of the laboratory performing the analysis to provide the appropriate supplies and ensure that they are lead-free. The supplies can include a 1,000-mL, acid-washed, plastic urine collection container and a 5- to 10-mL plastic tube or other device for transferring the urine sample aliquot and shipping it to the analyzing laboratory.

The procedure for sample collection is as follows:

(1) The patient excretes urine into a plastic, acid-washed container over an 8-hour period. (See Section 5.2.1.) Record the total volume (mL) of urine collected along with the dose of $CaNa_2EDTA$ (mg).

(2) Immediately after urine collection, mix well, and transfer a 5- to 10-mL aliquot into a plastic, leak-proof tube for transport to the analyzing laboratory. This may be easily accomplished using a purpose-designed plastic device for transporting samples for urinalysis.

(3) Urine samples may be transported to the laboratory at ambient temperature using approved (e.g., by the United States Post Office) shipping containers for etiologic agents.

(4) Upon receipt, urine samples should be refrigerated at approximately 4° C or frozen until the analysis can proceed.

5.3 Hair and Nails[12-14]

As biopsy materials, hair and nails have superficial appeal because they are easily collected and can be collected noninvasively. A naive assumption is that the trace element content of hair and nails reflects nutritional deficiency or toxic exposure occurring over the long term. However, the physiological significance of the trace element content of hair and nails is not well defined. Hair is particularly susceptible to contamination from the environment. Proposed procedures for removing surface contamination, such as shampoo residues from hair, include use of detergent solutions or alcohol-acetone mixtures. However, it is difficult to remove surface contamination while leaving intact the endogenous metal content.

The advantages and pitfalls of both hair and nails analyses for biological monitoring has been extensively reviewed.[15] In general, the problems of external contamination and lack

of good reference values make hair and nails analyses of extremely dubious value. Protocols are, therefore, not provided in this guideline.

5.4 Tissues

Trace element quantitation of soft tissues, in particular, the liver, is used in the diagnosis of disorders, such as hemochromatosis (iron) and Wilson's Disease (copper). Hard tissues, e.g., bone, may be used in the assessment of aluminum or lead poisoning.

The following factors must be considered when establishing a protocol for tissue collection: (1) selection of appropriate tissue (e.g., soft tissue, liver, kidney) or bone and (2) distribution of the element of interest within tissue.

Because tissue sample quantities vary with the biopsy protocol used, the laboratory should be consulted to determine the sample quantity that is sufficient for the analysis.

The typical minimum sample size for a needle-punch biopsy is 5 to 10 mm; for autopsy tissue, it is a 0.25 inch (6.4-mm) cube.

Selection of instruments used to obtain the tissue sample depends on the element of interest. Consideration should be given to the following concerns: (1) the use of stainless steel instruments that have been rinsed in EDTA[a] followed by Type I water and (2) that needle biopsy devices that extract a cylindrical piece of tissue or organ, such as the liver, must also be acid-washed.

For all types of tissue, including a bone wedge, the preferred storage container is a plastic, acid-leached, screw-capped vial or 50-mL, screw-capped, plastic centrifuge tube.

5.4.1 Biopsy Tissue

The usual procedure for a needle biopsy involves the use of a device that extracts a cylindrical piece of tissue or organ. The tissue should be immediately transferred to an acid-leached vial and transported to the laboratory or frozen and shipped to the laboratory on dry ice. Use of formalin and saline solutions must be avoided.

5.4.2 Collection

The procedure for collection is as follows:

(1) Use powder-free plastic gloves where possible or rinse the exterior powder from surgical latex gloves with deionized water.

(2) Collect tissue samples using accepted biopsy/surgical procedures.

(3) Rinse the tissue sample with deionized water (not saline) after collection and immediately place it in a polypropylene test tube. Do not add water, saline, or other liquid. Close the tube securely and send it to the laboratory.

(4) Alternatively, the tissue can be frozen and then shipped to the laboratory on dry ice. Avoid contact of the tissue with formalin or saline solutions.

5.5 Human Milk

In the establishment of requirements for infants, elemental analysis of human milk is used to assess element intake.

Before collecting the specimen, wash the mother's breast with deionized water. Express a small quantity of milk (approximately 1 mL) onto a gauze pad without allowing the nipple to touch the gauze. Discard the gauze and hand express about 15 mL of milk into the collection container. Do not touch the inside of the container.

Proceed by feeding the baby normally. At the end of the feeding, express and discard another 1 mL of milk. Express a second 15 mL of milk into the collection container. Close the container and refrigerate the specimen until the specimen is taken to the laboratory.

5.6 Stools

Twenty-four-hour stool collections should be weighed and then homogenized, if necessary,

[a] Disodium EDTA is prepared with Type I water to a concentration of 6 g/L.

with the addition of deionized water. The weight of the water added and the original weight of the stool should be recorded. A portion of the stool should be aliquoted into a plastic screw-capped vial for transport to the laboratory. Typically, sodium, potassium, and zinc have been determined on stools.

6 Specific Elements

General principles for the collection of samples for trace element analysis are suggested in Section 5. See Tables 1 and 2 for patient preparation considerations. This section provides a brief background discussion and emphasizes only specific variations of the previously defined protocols.

6.1 Aluminum

Indication: Monitoring aluminum accumulation/ toxicity in patients with renal failure, particularly those on hemodialysis treatment.[16]

Specimens: Serum (follow the collection protocol for ultratrace elements, Section 5.1.4), 24-hour urine, bone, special fluids (dialysate fluid, water).

Comments: Urine aluminum is used for occupational exposure (aluminum welders). Anticipate urinary excretion up to 100 times normal. The urinary concentration correlates with current air levels and number of years of exposure. Because this type of aluminum exposure has not been shown to be hazardous, the value of urinary monitoring is debatable.

Bulk analysis of ashed bone, or histochemical localization of aluminum at the mineralization front, provide an excellent means of assessing the body burden of aluminum to assess aluminum toxicity in patients with chronic renal failure.[17]

Reference Intervals[16]:

Plasma <10 μg/L (<370 nmol/L).
Urine 7 μg/24 h (259 nmol/d).
Tissue 0-2 μg/g dry weight (0–74 nmol/kg).

Conversion Factors (mass/mole):

μg/L x 37.06 = nmol/L.
nmol/L x 0.02695 = μg/L.

Patient preparation: Limiting oral ingestion of fruit juices and tea 24 h before blood collection is recommended because oral citrate enhances gastrointestinal aluminum absorption,[18,19] which results in increased blood concentrations.

6.2 Arsenic

Indications: Assessing arsenic poisoning, acute or chronic.

Specimens: 24-hour urine (collection protocol, Section 5.2.1).

Comments: Arsenic determinations in serum, plasma, or whole blood are of little value because the half-lives of arsenic species in blood appear to be short. A 24-hour urine collection is the specimen of choice.

If significant exposure or intentional exposure involving foul play is suspected, a random urine specimen collected in an office and submitted to the laboratory can document the presence or absence of arsenic.

Reference Intervals [20-21]:

Minimal seafood consumption <120 μg/24h (<1.60 μmol/d).

Heavy seafood consumption >120 μg/24h (>1.60 μmol/d).

Heavy seafood consumption can produce As levels in the 200 to 1000 μg/24h range.

Conversion Factors (mass/mole):

μg/24 h x 0.01335 = μmol/d.
μmol/d x 74.92 = μg/24h.

Patient Preparation: The patient should not consume seafood for several days before the collection.

Urine collection should be arranged away from the suspected exposure site. If workplace

exposure is suspected, the collection should be taken during off hours away from the work environment, preferably on the weekend.

6.3 Cadmium

Indication: Assessing industrial exposures.

Specimens: Random urine, whole blood (follow collection protocol for ultratrace elements, Section 5.1.4).

Comments: Between 10 to 50% of inhaled cadmium is absorbed, while only 5% of ingested cadmium is absorbed from the gastrointestinal tract. Gastrointestinal absorption is increased in iron and calcium deficiencies. The average amount ingested in most European and North American countries is about 10–20 μg/day (0.09–0.18 μmol/d). The half-life of cadmium in the body is 10 to 30 years.

Most cadmium in blood is in the erythrocytes. Smokers have twice the blood cadmium concentration seen in nonsmokers.

Because cadmium can be present in steel and in added anticoagulants, (e.g, heparin, citrate, and EDTA) the cadmium content must be checked before choosing a particular collection device or container.

The presence of increased amounts of low-molecular-weight proteins (e.g., β^2-micro-globulins) in urine can be used as an early indicator of exposure.

Reference Intervals:

Urine	0.5-1.0 μg/24 h (4–9 nmol/d).
Whole blood	(smokers) 1–4 μg/L[20,21] (8.9–35.6 nmol/L; 0.009–0.03 μmol/L)
	(nonsmokers) 0.3–1.2 μg/L[22,23] 0.003–0.011 μmol/L).

Generally, in long-term, low-level exposure, a urinary cadmium concentration above 10 μg/L or 10 mg/kg creatinine generally signals impending or actual renal tubular impairment. A blood value above 10 μg/L (0.089 μmol/L) implies that cadmium exposure of a significant degree has taken place.

Conversion Factors (mass/mol):

μg/L x 0.0089 = μmol/L.
μmol/L x 112.4 = μg/L.

Patient Preparation: Urine samples must be collected outside the industrial environment, i.e., in the home or a doctor's office. An early-morning urine sample is favored for sufficient concentration of cadmium.

6.4 Chromium

Indications: Assessing industrial exposure, and identifying workers who fail to adhere to recommended working practices.

Specimen: Serum (follow the collection protocol for ultratrace elements, Section 5.1.4), urine.

Comments: 24-hour-urine collection is the preferred biological specimen for biological monitoring purposes.

Blood and serum chromium concentrations are increased in workers exposed to chromium.

Reference Intervals:[1,24]

Serum	0.04–0.39 μg/L (6.769–7.50 nmol/L).
Urine	0.05–0.58 μg/L (0.962–11.15 nmol/L).

Conversion Factors (mass/mol):

μg/L x 19.23 = nmol/L.
nmol/L x 0.052 = μg/L.

Patient Preparation: There are no specific recommendations. For occupational exposure, specimen collection should be done away from the workplace, preferably over the weekend.

6.5 Cobalt

Indications: Chronic cobalt toxicity—occupational, identifying workers who fail to adhere to recommended working practices.

Specimen: Serum (follow the collection protocol for ultratrace elements, Section 5.1.4).

Comments: Chronic cobalt poisoning can manifest as cardiomyopathy; lung, skin, or gastrointestinal tract symptoms; hematological disorders; and thyroid disease.

Reference Interval: [25,26]

Serum 0.0394 - 0.271 μg/L (0.669 - 4.60 nmol/L).

Conversion Factors (mass/mol):

μg/L x 16.97 = nmol/L.
nmol/L x 0.059 = μg/L.

Patient Preparation: Avoid beer for 24 hours.

6.6 Copper

Indications: Diagnosis of (1) acquired copper deficiency (e.g., malabsorption, malnutrition, total parenteral nutrition), (2) genetic copper deficiencies (e.g., Menkes disease), (3) acquired toxicity/occupational exposure (smelting or from the production or use of pesticides, fungicides, or algicides), (4) accidental or intentional ingestion, and (5) genetic copper toxicity (e.g., Wilson's Disease).

Specimens: Serum, 24-hour urine, tissue (liver).

Comments: Serum is used to assess a person's copper status. Diurnal variation occurs, with the highest copper levels observed in the morning. Hypercupremia occurs in liver disease, infection and inflammation, trauma, or certain neoplasms. Oral contraceptives elevate copper levels by increasing ceruloplasmin concentration.

Because ceruloplasmin binds 0.3 μg Cu (0.0047 μmol; 4.7 nmol) per mg ceruloplasmin, ceruloplasmin-bound copper should approximate 90% of total copper and non-ceruloplasmin (if < 10 μg/dL) copper should be 1.57 μmol/L:

Non-Ceruloplasmin Copper [μmol/L] = *Total Copper* [μmol/L] - *Ceruloplasmin* [mg/L) x 0.0047.

A 24-hour urine excretion is used in diagnosing or assessing treatment for Wilson's disease. The level of copper found in the urine specimen after a penicillamine challenge is used in the diagnosis of Wilson's disease.

Copper found in liver tissue may be quantitated in the assessment of Wilson's disease or childhood cirrhosis in persons of American Indian descent.

Reference Intervals[b]:

Table 4 lists age-specific reference intervals for serum copper.[27,28]

Table 4. Age-Specific Reference Intervals for Serum Copper

Age	Sex	μg/dL	μmol/L
0–3 m	M/F	9-46	1.4-7.2
4–6 m		25-110	3.9-17.3
7 m–1 y		50-130	7.9-20.5
1–5 y		80-150	12.6-23.6
6–9 y		83-136	13.2-21.4
10–13 y	M	80-121	12.6-19.0
14–19 y		64-117	10.1-18.4
10–13 y	F	82-120	12.9-18.9
14–19 y		72-160	11.3-25.2

M, male; m, month; F, female; y, year.

Liver: < 50 μg/g dry weight. Normal hepatic copper concentration in neonates is significantly higher.

Urine: 24h: < 0.6 μmol/day (< 38 μg/day)[29];

Conversion Factors (mass/mol):

[b] Gestation-specific reference intervals are required in pregnancy.[30]

μg/dL x 0.1574 = μmol/L.
μmol/L x 6.353 = μg/dL.

Patient Preparation: To eliminate the effects of diurnal variation, collect samples at the same time each day.

6.7 Iron

Indication: Diagnosis of iron deficiency and anemia, iron toxicity, acute or chronic, hemochromatosis.

Specimens: Serum (assess deficiency or toxicity), 24-hour urine (monitor chelation therapy), liver biopsy (diagnosis of hemochromatosis).

Comments: Assessment of iron deficiency and overload might best be made by a combination of iron, transferrin saturation, and ferritin. Transferrin saturation of >80% is expected in hemochromatosis.

Reference Intervals[c]:

Liver < 290 μg/g (5.2 mmol/kg) dry weight (adults); Normal hepatic iron concentration in neonates is significantly higher. Contact reference laboratory before requesting this test.

Table 5 lists age-specific reference intervals for serum iron.[26,28]

Table 5. Age-Specific Reference Intervals for Iron

Age Group	*n*	μg/L	μmol/L
1–5	44	223–1396	4-25
6–9	50	391–1396	7-25
Males			
10–14	31	279–1340	5-24
14–19	65	335–1620	6-29
Females			
10–14	40	447–1452	8-26
15–19	110	279–1843	5-33

[c] Gestation-specific reference intervals are required in pregnancy.[30]

Conversion Factors (mass/mol):

μg/L x 0.0179 = μmol/L.
μmol/L x 55.84 = μg/L.

Patient Preparation: To eliminate the effects of diurnal variation, collect fasting morning serum samples at the same time each day.

After a patient undergoes a blood transfusion, delay specimen collection for at least 24 hours.

6.8 Lead[31-33]

Indications: Diagnosis of occupational or environmental lead exposure and screening for excess lead exposure in children.

Specimen: Whole blood and urine (calcium disodium EDTA mobilization test).

Comments: Generally, it is accepted that whole blood is the most reliable index of recent exposure to lead (BPb). For diagnostic purposes, venous blood should be analyzed, but for pediatric screening purposes, a capillary liquid blood lead level obtained using the fingerstick method is acceptable.

Reference Intervals:

Current studies conducted in the United States indicate that the geometric mean BPb level in the general population has fallen to around 2.8 μg/dL (0.14 μmol/L) and to around 3.6 μg/dL (0.17 μmol/L) in children.[34] For public health purposes, BPb concentrations of10 μg/dL (0.48 μmol/L) and greater, especially in children are considered to be lead poisoning.

There is a paucity of up-to-date published values for normal UPb levels. A 1988 survey of literature data reported a mean value for Pb in urine of 11 μg/L (range 6.3–13.0).[35] More recent normal mean urinary Pb levels, primarily from European Community populations, have been reported as 14.6 μg/L[36] and 17 μg/L.[37] In a recent Belgian study, the mean (geometric) normal urinary Pb excretion was reported to be 7.5 μg/g creatinine.[38] Multiplying this by 1.49 mg creatinine per day, the mean value for

creatinine excretion,[39] yields an excretion rate of 11 μg Pb/day.

Conversion Factors (mass/mol):

μg/dL x 0.04826 = μmol/L.
μmol/L x 20.72 = μg/dL.

Patient Preparation: The only preparation required is to ensure that the site of collection is properly cleaned before puncture. This is especially important when collecting blood for screening purposes using the fingerstick method, because contamination errors can be large (see recommended procedure for collecting blood, Section 5.1.5.)

For a lead mobilization (provocation) test, special collection instructions recommend collecting an 8-hour urine sample. This 8-hour urine sample is collected immediately after administering to the patient a dose of $CaNa_2EDTA$.

Note that all precautions described in the urine collection protocol (Section 5.2) should be followed.

6.9 Manganese

Indication: Industrial exposure.

Specimen: Serum.

Comments: Packed blood cell levels of manganese are approximately 25 times higher than plasma levels. Therefore, partially hemolyzed blood samples will yield plasma samples contaminated with intracellular manganese.

Reference Intervals:

Serum: 0.44 - 0.76 μg/L (8-13.8 nmol/L).[40]

Conversion Factors (mass/mol):

μg/L x 18.2 = nmol/L.
nmol/L x 0.055 = μg/L.

Patient Preparation: Obtain the specimen away from the workplace, preferably over the weekend.

6.10 Mercury

Indications: Assessment of mercury exposure.

Specimens: Whole blood, 24-hour urine.

Comments: The selection of urine or whole blood is dependent on the species of mercury to be assessed.

In the assessment of exposure to inorganic mercury compounds or salts, urine is the most suitable specimen for analysis. Inorganic mercury compounds or salts are weakly absorbed by the gastrointestinal tract, and they are rapidly eliminated from the blood to the kidney and liver. The urinary elimination route is favored.

To assess organic mercury compounds, whole blood is the most suitable specimen for analysis. For methyl- and ethyl-mercury-type compounds, the blood half-life is significantly longer than for inorganic compounds. Methyl-mercury is rapidly absorbed in the gastrointestinal tract and it accumulates in the red blood cells. The organic compounds circulate in the blood for a long time and they gradually accumulate in the central nervous system.

Reference Intervals:

Whole Blood: 0.6-59 μg/L (2.99-294 nmol/L; 0.003-0.294 μmol/L).

Urine: 0.1-20 μg/L (0.5-100 nmol/L).

Total mercury:

Blood: < 5 μg/L (25 nmol/L).
Urine: < 20 μg/24 h.

Conversion Factors (mass/mol):

μg/L x 4.985 = nmol/L.
nmol/L x 0.2004 = μg/L.

Patient Preparation: None required.

6.11 Molybdenum

Indication: Assessment of occupational exposure and assessment of acquired deficiency.

Specimens: Serum (follow collection protocol for ultratrace elements, Section 5.1.4), 24-h urine.

Reference Intervals [25,26]:

Serum: 0.19 - 1.16 μg/L (1.98 - 12.09 nmol/L).

Urine: 11.1 - 88.0 μg/24h (115.7 - 917.0 nmol/24h)

Conversion Factors (mass/mol):

μg/L x 10.42 = nmol/L.
nmol/L x 0.096 = μg/L.

Patient Preparation: Obtain the specimen away from the workplace, preferably over the weekend.

6.12 Nickel

Indication: Occupational monitoring, identifying workers who fail to adhere to recommended working practices.

Specimens: 24-hour urine, serum (follow the collection protocol for ultratrace elements, Section 5.1.4).

Comments: Nickel concentrations in urine, or serum specimens taken from workers with inhalation exposures to soluble nickel salts, reflect primarily the amount of nickel absorbed during 1 or 2 preceding days. For less soluble nickel compounds, nickel concentrations in serum or urine reflect the combined influences of recent exposures.

Nickel concentrations in urine and the serum of workers in a nickel refinery have been found to be significantly correlated.[41]

Reference Intervals[42]:

Serum: < 2.0 μg/L (0.034 μmol/L)

Urine: 0.6 μg/24 hrs (0.010 μmol/d).

Conversion Factors (mass/mol):

μg/L x 0.017 = μmol/L.
μmol/L x 59.1 = μg/L.

Patient Preparation: Obtain the specimen away from the workplace, preferably over the weekend.

6.13 Selenium

Indications: Assessment of selenium deficiency or toxicity.

Specimen: Serum or plasma.

Comments: Only low selenium concentrations in serum correlate well with glutathione peroxidase status. It appears that whole blood selenium analysis is of little value.

For determining selenium in urine, a 24-hour urine collection is recommended.

Reference Intervals[d]:

Table 6. Age-Specific Reference Intervals for Plasma or Serum Selenium in British Columbia

Age	μg/L	μmol/L
Preterm	35–94	0.44–1.19
Term	57–96	0.72–1.21
1-5 years	96-144	1.22-1.82
6-9 years	101–162	1.28–2.05
10-16 years	103–186	1.31–2.35
Adult	109–181	1.38–2.29

Age-specific reference ranges must be used. Ranges may vary by geographical location. As an example, Table 6 lists the reference intervals for plasma or serum selenium in British Columbia.[27,43]

Conversion Factors (mass/mol):

μg/L x 0.0127 = nmol/L.

[d] Gestation-specific reference intervals are required for pregnancy.[30]

nmol/L x 78.96 = μg/L.

Patient Preparation: None required.

6.14 Uranium

Indications: Occupational monitoring, and identifying workers who fail to adhere to recommended working practices.

Specimens: 24-hour urine.

Comments: To comply with U.S. Nuclear Regulatory Commission (NRC) regulations 8.11 and 8.22, uranium (U) must be determined in the urine of workers at U.S. nuclear facilities. Based on the U concentration in urine, metabolic models are used to estimate the body burden of U in the kidneys and lungs of persons exposed to ingestible and respirable U dust.

The action levels recommended for protection of uranium workers are 100 μg/L of U excreted during the first 24 h after an acute exposure and, for chronic exposure, 1 μg/L of U for the first excretion on Monday morning.[44]

The analytical requirement for U suggested by the NRC is determination at the 100-ng/L level in a 10-mL sample with 10% uncertainty.

Reference Intervals:

12 ng/L to 26 μg/L[45] (0.05 nmol/L to 0.109 μmol/L).

Conversion Factors (mass/mol):

μg/L x 4.2 = nmol/L.
nmol/L x 0.238 = μg/L.

Patient Preparation: None required.

6.15 Vanadium

Indication: Vanadium (V) is considered an essential ultratrace element for animals, but there is no conclusive evidence that it is essential for humans.[46,47] It has been reported to inhibit a number of enzyme activities, such as those of Na^+-K^+-ATPase, the sodium potassium pump[48] and, because it is excreted primarily via the kidney, it tends to accumulate in patients on hemodialysis.[49]

Specimen: Serum for assessment of V status. Urine vanadium for biological monitoring of chronic occupational exposure.

Reference Intervals:

Serum:	
Adults[49,50]	<0.08-2.4 μg/L (<1.6–47 nmol/L).
Male[50]	0.029-0.939 μg/L (0.57–18.4 nmol/L).
Female[50]	0.017-0.053 μg/L (0.33–1.04 nmol/L).

Normal serum concentrations: <1.0 μg/L (<19.6 nmol/L).

Conversion Factors (mass/mol):

μg/L x 19.6 = nmol/L.
nmol/L x 0.051 = μg/L.

Patient Preparation: No special preparation required.

6.16 Zinc

Indication: Suspected zinc deficiency (inherited or acquired) or toxicity.

Specimen: Serum, urine (toxicity).

Comments: Hemolysis increases apparent concentration.

Reference Intervals[e, 25, 26]

Serum:	70.0-120.0 μg/dL (10.7-18.4 μmol/L).

[e] Gestation-specific reference intervals are required for pregnancy.[30]

Table 7 gives age-specific reference intervals for serum zinc.[27,28,41]

Table 7. Age-Specific Reference Intervals for Serum Zinc

Age	Sex	μg/dL	μmol/L
0–1 d	M/F	65–81	9.9–12.4
2 d–1 y		65–130	9.9–19.9
1–5 y		67–118	10.3–18.1
6–9 y		77–107	11.8–16.4
10–14 y	M	76–101	11.6–15.4
15–19 y		64–117	9.8–17.9
10–14 y	F	79–118	12.1–18.0
15–19		60–101	9.2–15.4

M, male; d, day; F, female; y, year.

Conversion Factors (mass/mol):

μg/dL x 0.153 = μmol/L.
μmol/L x 6.537 = μg/dL.

Patient Preparation: To avoid the effects of diurnal variation, collect a morning fasting specimen.

Background: After iron, zinc is the most abundant trace metal in the body and, as such, the normal concentrations of zinc in biological samples is relatively high. Zinc is an essential component of many important enzymes, including alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, procarboxy peptidase, and superoxide dismutase.

The most common forms of zinc deficiency are from malnutrition, excessive zinc excretion, or malabsorption. Stress and tissue destruction from severe burns, surgery, or trauma can also cause zinc deficiency.

Zinc toxicity can result from industrial exposure to dust or metal fumes. Consumption of acidic food and beverages packaged in galvanized cans has also been reported as a source of excess zinc exposure.

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Summary of Comments and Subcommittee Responses

C38-P, Control of Preanalytical Variation in Trace Element Analysis; Proposed Guideline

General Comments

1. The inconsistencies between tables 1, 2 and 3, and the Specific Elements, Section 6 and following should be addressed. For example, cobalt in table 1 recommends, "avoid beer for 24 hours" and in Section 6.5 cobalt lists nothing in patient preparation or comments about avoiding beer.

- **The guideline has been revised to ensure consistency.**

Section 4.3.2

2. Clearly ASTM does not consider water 10 MΩ/cm to 16.6 MΩ/cm to be Type I! Additionally, the Geigy Scientific Tables (Vol. 1, 8th ed., Ciba-Geigy 1981) recommend SI unit of resistivity to be Ωm.

- **Section 4.3.2 has been edited to eliminate any confusion with respect to minimum resistivity.**

Section 5.1.2

3. How do you "ream" an anticoagulated specimen?

- **This statement has been deleted from Section 5.1.2.**

Section 5.1.4

4. Column 2, under "**Note**:" "If certain syringes..." Would this not be clarified by rephrasing as: "If noncentrifugable syringes..."?

- **The note has been reworded to clarify its intent.**

Section 5.2.2

5. I'm not sure a 24-hour urine collection is "always preferable," because 24-hour urines, even in a hospital setting, often really do not span 24 hours, and are much more prone to contamination than a single void into a precleaned container that is only opened once. There is literature data suggesting that urinary protein/creatinine ratio more accurately reflects renal protein excretion than 24-hour proteins because of collection timing errors. Thus, I would (reword) the first phrase in this section "a urine..."

- **Although the subcommittee believes a 24-hour urine specimen is preferable, it agreed that there are instances when a random urine may be sufficient for screening purposes. The "always" qualifier has been deleted.**

Section 5.4

6. 'Tissues,' paragraph 4, line 3 "...a 1.25 inch (6.4-mm) cube." This should be "...an 0.25 inch (6.4-mm) cube."

- **This has been corrected in C38-A.**

7. The subcommittee has done an excellent job with this very handy guideline. Paragraph 5 of this section states, "the use of stainless steel instruments that have been rinsed in EDTA followed by deionized water." The concentration and make up of the EDTA should be specified.

- **A footnote has been added to Section 5.4 that states, "Na_2 EDTA is prepared with Type I water to a concentration of 6 g/L."**

8. Some consideration for toxic concentrations in the reference intervals for toxic metals (e.g., arsenic, aluminum, cadmium, lead, mercury, etc.) should be given. Having dealt with several cases of suspected and at least one case of actual (arsenic) poisoning, this information is hard to get from the literature.

- **The subcommittee does not believe there are sufficient published data to support establishing reference intervals for toxic concentrations of trace elements.**

Section 6.1

9. 'Aluminum', paragraph 2 'Specimens' — you suggested plasma as the specimen type while at the same time indicating that the collection procedure for ultra trace elements be followed. Section 5.1.4, Ultratrace specimen collection, results in serum.

   Even if one desired to collect blood for plasma through a Teflon® catheter, it would likely produce plasma with unacceptably inconsistent aluminum values due to variable anticoagulant contamination. The most aluminum-free lithium heparin tubes we found (by no means do I suggest we've tested all the available products!) contributed a mean aluminum concentration to plasma samples of 2.5 μg/L (range 1.4 - 3.3 μg/L) when that exact procedure was tested (CD Hewitt, et al, Critical Appraisal of Two Methods for Determining Aluminum in Blood Samples, Clin. Chem., 1990;36:1466-9). This variation is acceptable for aluminum analyses on samples of patients undergoing renal dialysis with elevated aluminum [trace level concentration] but would be approximately 20% of the aluminum content of a sample from a patient with normal [$<$10 μg/L; ultratrace] levels. Therefore, we believe that the preferred blood specimen, and the only one for which a Teflon® catheter would reliably be of benefit, is serum— when collecting a sample for ultratrace [normal] aluminum levels.

- **The subcommittee agrees that serum is the appropriate specimen for aluminum. C38-A has been corrected.**

Section 6.2

10. Recommend clarifying the intent of the second paragraph under "comments." I think the message the writers of the document intend to say "if significant or intentional exposure involving foul play are suspected..." I know of several cases where the intentional poisoning

was being done by a relative other than a spouse or a nonrelative (e.g. boyfriend, girlfriend, etc.).

- **The paragraph has been reworded to state, "If significant exposure or intentional exposure involving foul play is suspected, a random urine specimen collected in an office and submitted to the laboratory can document the presence or absence of arsenic."**

Section 6.3

11. Last paragraph of "comments." Delete electrophoretic since immunoassays for specific proteins (e.g. ß-2-microglobulin) are better tests.

- **The statement was revised to read, "Assay of low-molecular-weight proteins (e.g., β^2-microglobulins) in urine can be used as an early indicator of exposure."**

Section 6.6

12. In Table 4 (page 17), age 6-9 years, the range should be "83-136" or "84-136" but not "834-136."

- **The correction, "83–136," has been made.**

Section 6.7

13. Considering the brevity of this section compared to the complexity of measuring body iron stores, both deficiency and excess, I wonder if iron should be included in this document. Although the authors suggest the upper limit of concentration for a trace element as 10,000 μg/L, citing their reference,[1] I don't think most clinical chemists consider iron a "trace" element. The authors might consider dropping section 6.7, although I do not have strong feelings on this issue.

- **The subcommittee concluded that, for completeness, iron should remain in the guideline. References have been added for readers requiring further information.**

14. In table 5, it is not clear where the referenced values originate. I would suggest including age-specific reference intervals (5-95th percentile) as provided in NCCLS document H17-P (page 12) which include a further age subdivision and were obtained from the HANES program 1971-1980.

- **References have been added.**

Section 6.9

15. Under the comments section, if the second sentence of the second paragraph is true, how can adult levels be referenced as 22-23 times the infant level?

- **The guideline has been corrected so that the reference intervals are correctly labeled.**

Section 6.12

16. Two intervals are given for serum nickel, less than 2.0 μg/L and 2.6 - 7.5 μg/L. Only one of these can be the correct one; which one?

- **The reference intervals have been revised and a supporting reference added.**

17. The subcommittee should seriously consider "folding in" H31-P (containers for toxicological analysis) into this document, e.g., as an appendix.

- **The subcommittee does not support this recommendation as the guideline H31-P is broader in scope than trace elements.**

Related NCCLS Publications[‖]

C3-A3 **Preparation and Testing of Reagent Water in the Clinical Laboratory—Third Edition; Approved Guideline** (1997). C3-A3 addresses the requirements for purified water, methods for monitoring quality and testing for specific contaminants, and system-design considerations.

GP16-A **Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline** (1995). GP16-A discusses procedures that address materials and equipment, macroscopic examinations, clinical analyses, and microscopic evaluations. Also, the document offers information on collection, specimen criteria, and storage.

H3-A3 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture—Third Edition; Approved Standard** (1991). H3-A3 discusses methods of collection, as well as a training program for increasing integrity and for minimizing error.

H4-A3 **Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture—Third Edition; Approved Standard** (1991). H4-A3 describes proper collection techniques and discusses hazards to patients.

H14-A2 **Devices for Collection of Skin Puncture Blood Specimens—Second Edition; Approved Guideline** (1990). H14-A2 gives specifications of disposable devices for collecting, processing, and transferring diagnostic blood specimens obtained by skin puncture.

H18-A **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline** (1990). H18-A addresses the multiple factors involved in the handling and processing of specimens that can introduce imprecision or systematic bias into results.

H24-T **Additives to Blood Collection Devices: Heparin; Tentative Standard** (1988). H24-T contains a technical description of heparin compounds used in devices. The document also addresses evaluation of the suitability of heparin-containing devices and the quantitation of heparin.

H31-P **Collection Containers for Specimens for Toxicological Analysis; Proposed Guideline** (1986). H31-P discusses the recommended toxicology/drug monitoring requirements.

H35-T **Additives to Blood Collection Devices: Edta; Tentative Standard** (1992). H35-T offers a technical description of ethylenediaminetetra-acetic acid (EDTA) and its use in blood collection products.

[‖]Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.