Pure & Appl. Chem., Vol. 67, Nos 8/9, pp. 1575–1608, 1995.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

CLINICAL CHEMISTRY DIVISION
COMMISSION ON TOXICOLOGY* WORKING PARTY†

SAMPLE COLLECTION GUIDELINES FOR TRACE ELEMENTS IN BLOOD AND URINE

(Technical Report)

Prepared for publication by

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Sample collection guidelines for trace elements in blood and urine

Synopsis

This paper presents an organized system for element-specific sample collection and handling of human blood (whole blood, serum or plasma, packed cells or erythrocytes) and urine also indicating a proper definition of the subject and sample. Harmonized procedures for collection, preparation, analysis and quality control are suggested. The aim is to assist scientists worldwide to produce comparable data which will be useful on a regional, national and international scale. The guidelines are directed to the elements aluminium, arsenic, cadmium, chromium, cobalt, copper, lead, lithium, manganese, mercury, nickel, selenium and zinc. These include the most important elements measured for their occupational or clinical significance, and serve as examples of principles that will guide development of methods for other elements in the future.

INTRODUCTION

The IUPAC Commission of Toxicology is preparing recommendations for analytical quality criteria in biological monitoring of toxic elements. This includes the study of environmental exposure - either to establish reference ranges or to examine a specific problem - as well as occupational biological monitoring. Investigation of a person, e.g. with symptoms suggestive of undue trace element exposure, is also within the scope. For this purpose a working party was set up consisting of 16 members whose names are listed above. This working party intends to give guidelines for defining the subject, and to suggest both general and element-specific guidelines for the harmonized assessment of aluminium, arsenic, cadmium, chromium, cobalt, copper, lead, lithium, manganese, mercury, nickel, selenium and zinc, that will cover sample collection, preparation in relation to analytical methodology, quality assurance and evaluation of the data. Specific guidelines for the selected elements are presented. They include expected concentrations. Recently very stringent criteria for evaluating literature data have been advocated by Vesterberg et al. in their TRACY project, an evaluating system rating published data according to a detailed check-list [1,2].

In addition to guidelines on sample collection, lists of criteria are given in appendix 1 that are intended to define

A: Subject history
B: Sample collection
C: Post-sampling steps.

It is suggested that answers to questions in part A be completed by the physician, the nursing staff or the researcher and not written on a form by the subject. This will favour a more accurate reply and allow a more precise definition of the subject.

A. SUBJECT HISTORY

A detailed description of the subject is a first prerequisite to allow for classification in a particular group or "universe" on the basis of well defined criteria for purposes of comparison. It is not easy to develop guidelines regarding the personal history of a subject. Such guidelines depend on the kind of element to study and on the kind of study to be performed. For studies regarding dose-effect or dose-response relationships, other parameters are of greater importance than in case of reference values. Moreover, differences exist among occupational health, environmental health and clinical studies. For more detailed studies the researchers will obviously have to design more specific questionnaires. It has been the goal of the working party to identify, with the benefit of hindsight, some important factors that will determine whether the results of the investigations will apply to the same reference

populations. The Commission on Toxicology realizes that research on trace element concentrations in biological fluids and tissues may yield many different "reference" values, each of which will apply only to a particular group. If sufficient detail is published, such values maybe useful for comparative purposes.

The description starts with information about the identity of the subject, stipulation of where and when the sampling takes place, and the identity of the medical staff collecting the samples. Each sample must bear a unique code value, which may be the name and identification number of the person.

The personal history of the subject may need to be quite elaborate. Details may be requested about the ethnic origin, sex, date of birth, recent and past employments, working environment, smoking habits and hobbies, not only of the person to be sampled but also of other family members, in order not to overlook hidden exposures. For example the concentration of lead in blood of children may be influenced by the occupation of the father [3]. It has been assumed that the father carries lead into the house on dirty work cloths. For certain studies in environmental and occupational health it may be important to obtain some information on the social status of the subject.

Descriptions of eating and drinking habits may sometimes be relevant, to allow an estimate of the amount of the element consumed with food and drink. This is, however, dependent on the study design and element of interest. In cases of As and Hg, it is appropriate to report estimated amounts and perhaps also the different varieties of fish consumed. Some fish varieties contain several mg/kg As as arsenobetaine, that is non-toxic and mainly excreted in urine. Fish in many areas may contain about 1 mg/kg of mercury mainly in the form of methylmercury, which increases concentrations in erythrocytes. The intake is excreted over a period of weeks, mainly via faeces and only slightly in urine.

The region of residence of the subject can affect some trace-element concentrations in the human body. For example, living in urban areas increases the blood Pb content, and living in the vicinity of factories handling Cd may cause increased Cd concentrations.

The personal questionnaire is completed with a detailed medical history of the subject, based on an interview with medical and/or research personnel, supplemented by the written medical record.

B. SAMPLE COLLECTION

A great number of serious errors may be introduced during the whole range of procedures from sample collection to the ultimate detection of the analyte. The following text is meant to generate a sound way of thinking when embarking on trace element measurements in biological fluids. The simplified list of recommendations is not exhaustive, and it is hoped that they can thereby be realized in normal laboratory practice. No claim is made that by applying these simple rules the ultimate state of perfection will be reached.

Blood

1. Equipment and cleaning procedures

1.1. Collection tube

A collection tube completely free of trace element contaminants, as claimed by some manufacturers, simply does not exist. The raw material used in manufacture as well as the impurities present will always release some atoms to the sample. The amount of the trace element contributed by the cleaned tube, including the stopper, to the blood or its derivatives should be low and exactly measured so that its contribution to the blank value can be reported. Suggestions for avoiding certain materials during collection of samples for analysis of specific elements are quite straightforward. For example, glass should not be used for collection of serum for Al measurement, plastics with a Cd-based softener are to be avoided when sampling for Cd measurement, Zn-doped stoppers should not be used when Zn is to be measured, etc.

In most cases evacuated blood collection tubes are used for blood sampling. These cannot be washed and their use for trace element determinations should be validated in each specific study. Ultimately the analyst, often in co-operation with the health-care personnel, carries the responsibility for testing the sampling tubes. A reliable testing procedure consists of analysing a certified sample of blood or plasma, after storage in the tube for a length of time, typical for the study, and containing a very low concentration of the element. If the certified value is produced the contamination can be considered

negligible. This implies that the analytical procedure is under control, which again can only be confirmed by the analysis of a blood sample certified for about the same concentrations of the trace element as found in the real samples. There are, however, very few if any elements certified in blood samples at the concentrations found in the general population in "low-exposure" areas. This testing procedure is not applicable in such cases, and an alternative method is needed.

An option for testing the collection tube consists of a rinse with mild acid, followed by analysis of the leaching solution. However, this procedure is largely irrelevant to the analysis of a body fluid. On the one hand it is too strongly acidic and on the other fails to subject the apparatus to the many ligands present in the biological fluids - notably aminoacids and peptides - that frequently have significant affinity constants with trace elements present on the wall or in the material of the tube.

To illustrate the possible impact of contaminations from the tube wall, the Mn contamination measured in serum collected in unwashed vials can amount to more than 100%. After a single rinse another 10% may still be added. In contrast, vials cleaned thoroughly yield an expected value of $1 \cdot 10^{-8}$ mol/L (0.55 ng/ml) [4]. Although Mn is an extremely difficult element to handle as far as avoidance of contamination is concerned, this example is typical of many transition elements.

Versieck and Cornelis [5] describe a very reliable cleaning procedure for blood collection vials and other laboratory ware in class 100 circumstances (class 100 means less than 100 particles of $> 0.5\mu m$ per cubic foot of air). The cleaning procedure encompasses, in part, leaching of the vials with pro analysis acids at increased temperature, rinsing with distilled water, followed by treatment with suprapure (1+10) HNO_3 and (1+10) H_2SO_4, rinsing with double-distilled water, and finishing with steam cleaning. These procedures are undoubtedly too elaborate and too expensive for routine laboratory work and are not necessary for all trace elements.

1.2. Needle

The use of a stainless steel needle for the collection of blood is generally not suitable. For measurement of some elements, e.g. for Cr, Co, Ni, Mn, an acceptable alternative is the use of a polypropene intravenous cannula, mounted on a trocar. The advocating of siliconized needles for trace element determinations may be very misleading, because some types of needles are only siliconized on the outside to facilitate their introduction into the artery. If so, the hazard of contamination of the blood sample remains a serious possibility.

1.3. Anticoagulant

The use of anticoagulants is very problematic, as most anticoagulants are either polyanions (e.g. heparin) or metal chelators (e.g. EDTA, citrate) and therefore have a high affinity for contaminating metal ions. This may be illustrated by analysis of Mn in serum and plasma where a totally unacceptable outcome has resulted [6]. In some cases (e.g. Zn) the problem is diminished, but as a general rule, this contamination hazard must be evaluated for each particular element under investigation, and the blank value must be reported for each batch of anticoagulant. Therefore, serum is generally to be preferred to plasma for measuring trace elements.

2. Separation of serum and packed cells

A recommended procedure for the separation of blood cells from serum, applicable to the analysis of either, is described in part B of appendix 1. The hazard of contaminating the blood sample with sweat from the skin of the subject or the hands of the physician may be significant in certain occasions. For example the Ni concentration in sweat is about 20 times, and the Cr concentration about 10 times that in serum [7]. Standardization of the clotting and separation procedures and careful avoidance of haemolysis are important. As some elements (e.g. Pb, Zn) have much higher concentrations in erythrocytes than in plasma, haemolysis should be assessed by measuring haemoglobin in the plasma or the serum sample when these elements are to be determined.

3. Storage

The sample should be stored either in a refrigerator at <5°C or frozen at -20°C or less (plastic tubes only). Time of storage, without changes occurring, may be element-dependent, although it can be anticipated that the analytical uncertainty for most elements is too high to discern any difference during reasonable periods of storage.

Urine

Depending on the purpose and the circumstances of the measurement, the investigator will choose between a 24 h urine collection or a spot sample. A strategy encompassing several spot samples collected at specified times, e.g. morning, noon, evening, may be easier to control than 24 h samples. An alternative may be to collect the urine voids every 3 hours. For studies involving the general population, a morning urine sample may be recommended, because the element concentration is then often relatively high. Information on the time of sampling and a timed record of the solid food and liquid intake may be necessary to interpret certain results. In occupational health monitoring the timing of collection in relation to the work shift depends on the biokinetics of the analyte, and is element-specific.

Measurement of the creatinine concentration and/or density is mandatory in all cases. Advantages and disadvantages with these measurements have been thoroughly discussed by Diamond [8] and Araki et al. [9]. Density measurement using a refractometer should be combined with a strip test for sugar, which will increase the density, e.g. in diabetics.

The urine samples may be stored in the presence of a preservative (e.g. HCl or HNO_3, depending on the method of choice). For some elements, such as Hg, a preservative with a high redox potential may be needed to ensure that the element of interest remains in the original oxidation state, if speciation analyses are to be undertaken,or loss of volatile reduction products (e.g. Hg^0) to be prevented. A preliminary check of the purity of the reagent is mandatory.

1. Equipment and cleaning procedures

A suitable collection vessel for urine is a polythene container, thoroughly cleaned according to procedures described above.

2. Urine collection

Although this may seem trivial, it is not so evident that the urine collected will provide a sample adequate for trace element determinations. Dust falling into the container is a potential hazard, as the cover must be removed for collection several times during the day. This is especially problematic for 24 h urine samples, because the collection vessel will be opened (usually in room air). Voiding of urine from the body into the vessel again introduces a major risk of contamination, from clothes and the skin.

Urine should be voided directly into an acid-washed polyethylene container that can be closed with an airtight lid. The subject should be instructed to minimize contamination of the sample by avoiding contact with the inside of the container or lid, and to refrain from leaving the container open to the air longer than necessary. In between sampling sessions, the container is wrapped in a clean polyethylene bag. It may also be recommended to collect each void in separate containers. For woman and young children the container-neck should have a diameter of about 12 cm. Timing of spot urines must be recorded and standardized within the study, e.g. first void of the day, pre-prandial, etc.. In general it is recommended to include measurement of the following parameters

parameter	
urinary density	usual range 1.012 - 1.030 (discard outlying samples)
creatinine	report concentration (only useful for 24 h collection, as requested for some elements, Al e.g.)
proteinuria	negative by strip test
UTI (urinary tract infection)	negative for nitrite producing bacteria
sugar	negative by strip test

3. Storage

Storage time at 4°C should be minimized, particularly in case of analysis of those elements that coprecipitate with other compounds. Depending on the type of analyses, the urine may be acidified with ultrapure concentrated HNO_3 or HCl(1'% v/v). Prior to sub-sampling for analysis, the sample should be shaken vigorously for 1 min to ensure a homogeneous suspension.

A stability study of 15 elements in urine over 3 days storage at room temperature revealed no significant differences for Co, Cu, Mn, Se, Zn, Br, Ca, Cl, Cr, Cs, I, K, Na and Rb [10]. On the other hand there appeared about a 15 % loss of As in the precipitate or on the walls.

C. POST SAMPLING STEPS

1. Information on sample treatment and analytical methodology

Preparation

Some or all of the following steps will have to be considered:
sample dilution, sample digestion or complete mineralization, filtration, deproteinization, preconcentration, solvent extraction, evaporation, hydride generation. All these steps should be recorded precisely, and the blank samples treated in exactly the same way as the authentic samples.

Measurement and calibration

It is beyond the scope of this article to discuss all the possible methods for the determination of trace elements. A method with adequate accuracy, specificity and sensitivity (LOD=limit of detection) must be chosen, and employed correctly so as to achieve these criteria.

2. Quality assurance

Nowadays most clinical laboratories are aware that their analytical procedures should be subject to the basic rules of quality control and quality assurance [11, 12, 13]. The internationally accepted definition and explanation of these terms can be found in the publication of the International Organization for Standardization [14]. The determination of reference materials of blood, serum, packed cells or urine, certified for trace elements at the same concentration as they are present in the samples, is a prerequisite to harmonize the results and provide quality assurance. A list of available reference materials for blood, its derivatives and urine are listed in Table 1.
It may be noted that most certified reference materials are available at one or a few concentrations only. It is strongly recommended that the analytical performance be verified by participation in external quality control programmes at regular intervals. Quality assurance of sampling and handling also implies the control of the analytical blank. Vahter [15]and the WHO [16] give important recommendations for quality assurance and quality control for biological monitoring of metals. No magic formula exists to assure the quality of the data. Continuous critical surveillance and quality control procedures that form an integral part of the monitoring project itself are major assets in this endeavour.
Certified reference materials (CRM) are not available for all 13 trace elements in blood, serum and urine that are considered. When a CRM is lacking, it is recommended that the laboratory produces a control material and establishes the element's concentration in the control material by inter-laboratory comparisons using, if possible, a different method. For example, human urine samples spiked with known amounts of arsenate, methylarsonic acid and dimethylarsinic acid, have been used for quality control purposes in the determination of As metabolites in urine, often used as an indicator of exposure to inorganic arsenic[17].

3. Evaluation of data

The evaluation of the data is dependent on the normal range of biological variability and on the end use of the results. A measurement process capable of producing the same value with 10% or even 20% accuracy would be considered to be precise enough for ultra-trace determinations (As, Co, Mn, Ni, ..), but not for Zn, Cu or Se. As a rule of thumb the accuracy and precision should be adequate with respect to the biological variation. Publications on concentrations of trace elements in biological media should always contain a detailed description of the analytical procedure. Particularly important is adequate documentation of the performance of the method, i.e. limit of detection, limit of quantification, linear range, repeatability, reproducibility, quality control, etc., with reference to previous IUPAC guidelines and to ISO 5725. Reference values and reference intervals should be calculated in accordance with IFCC (International Federation of Clinical Chemists) and IUPAC recommendations[18,19]

D. TYPICAL RANGES OF CONCENTRATIONS FOR TRACE ELEMENTS

Examples of values for many elements in plasma or serum can be found in the book by Versieck and Cornelis [5], for packed cells and urine by Versieck [20]. A series of review articles on trace element reference values in tissues from inhabitants of the European Community have been published

Table 1: Survey of reference materials of blood, serum and urine with certified and non certified concentrations of one or more of the elements Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se and Zn [34, 35]. (The concentrations are mostly atypical for unexposed individuals. Some products are expressed on a dry weight basis)

Material	Supplier Code number	Elements	Concentration	Error % C.I.	
Whole blood					
Bovine blood CRM	BCR-CRM-194	Cd Pb	0.0005 mg/L 0.126 mg/L	20 3.2	a a
Bovine blood CRM	BCR-CRM-195	Cd Pb	0.00537 mg/L 0.426 mg/L	20 3.2	a a
Bovine blood CRM	BCR-CRM-196	Cd Pb	0.0124 mg/L 0.772 mg/L	4.0 1.4	a a
Animal Blood CRM	IAEA-A-13	Cu Ni (not certified) Pb (not certified) Se Zn	4.3 mg/kg 1 0.18 0.24 mg/kg 13 mg/kg	13 40 44 33 7.7	a* a* a* a* a*
Blood CRM	KL-100-H	Pb	0.95 mg/L	21	o
Blood CRM	KL-100-L	Pb	0.2 mg/L	30	o
Blood CRM	KL-100-M	Pb	0.45 mg/L	22	o
Blood CRM	NIST-SRM-955a	Pb	0.057 mg/L	8.8	b
Blood CRM	NIST-SRM-955b	Pb	0.305 mg/L	1.0	b
Blood CRM	NIST-SRM-955c	Pb	0.494 mg/L	1.6	b
Blood CRM	NIST-SRM-955d	Pb	0.732 mg/L	1.0	b
Whole blood RM	NYCO-112	Cd (not certified) Hg (not certified) Pb (not certified)	0.0056 mg/L 0.004 mg/L 0.062 mg/L		
Whole blood RM	NYCO-904	Cd (not certified) Hg (not certified) Pb (not certified)	0.0124 mg/L 0.01 mg/L 0.374 mg/L		
Whole blood RM	NYCO-905	Cd (not certified) Hg (not certified) Pb (not certified)	0.019 mg/L 0.014 mg/L 0.788 mg/L		

Table 1 (continued)

Material	Supplier Code number	Elements	Concentration	Error % C.I.	
Serum					
Serum CRM	KL-146-I	Cu Zn	0.7 mg/L 1.3 mg/L	21 19	o o
Serum CRM	KL-146-II	Cu Zn	1.5 mg/L 1.2 mg/L	20 20	o o
Serum CRM	KL-147-I	Al Mn	0.35 mg/L 0.03 mg/L	17 33	o o
Serum CRM	KL-147-II	Al Mn	0.7 mg/L 0.04 mg/L	21 37	o o
Serum CRM	KL-148-I	Cr Ni Se	0.075 mg/L 0.03 mg/L 0.15 mg/L	80 66 40	o o o
Serum CRM	KL-148-II	Cr Ni Se	0.15 mg/L 0.05 mg/L 0.25 mg/L	40 40 24	o o o
Serum RM	NYCO-105	Al (not certified) Cu (not certified) Hg (not certified) Pb (not certified) Ni (not certified) Se (not certified) Zn (not certified)	0.07 mg/L 1.3 mg/L 0.0011 mg/L 0.0027 mg/L 0.0032 mg/L 0.09 mg/L 0.9 mg/L		
Serum RM	NYCO-112	Al (not certified) Cu (not certified) Se (not certified) Zn (not certified)	0.07 mg/L 1.1 mg/L 0.09 mg/L 0.8 mg/L		
Human Serum CRM	University Gent	Al As Cd Co Cr Cu Mn Se Zn	0.0202 mg/L 0.0196 mg/kg 0.0020 mg/kg 0.0036 mg/kg 0.00076 mg/kg 11.1 mg/kg 0.0077 mg/kg 1.05 mg/kg 9.6 mg/kg	14 20 20 16 13 3.6 3.9 4.8 4.2	a a a a a a a a a
Urine					
Urine CRM	KL-110-H	Pb	1.1 mg/L	18	o
Urine CRM	KL-110-L	Pb	0.15 mg/L	47	o

Table 1 (continued)

Material	Supplier Code number	Elements	Concentration	Error % C.I.	
Urine CRM	KL-110-M	Pb	0.5 mg/L	20	o
Urine CRM	KL-140-I	As	0.05 mg/L	25	o
		Cd	0.01 mg/L	25	o
		Hg	0.02 mg/L	25	o
Urine CRM	KL-140-II	As	0.15 mg/L	20	o
		Cd	0.03 mg/L	20	o
		Hg	0.06 mg/L	20	o
Urine CRM	KL-142-I	Cr	10 mg/L	50	o
		Ni	10 mg/L	50	o
		Se	20 mg/L	37	o
Urine CRM	KL-142-II	Cr	0.2 mg/L	37	o
		Ni	0.2 mg/L	37	o
		Se	0.5 mg/L	20	o
Urine RM	NYCO-108	Al (not certified)	0.161 mg/L		
		As (not certified)	0.2 mg/L		
		Cd (not certified)	0.0062 mg/L		
		Co (not certified)	0.011 mg/L		
		Cr (not certified)	0.022 mg/L		
		Cu (not certified)	0.045 mg/L		
		Hg (not certified)	0.051 mg/L		
		Mn (not certified)	0.02 mg/L		
		Ni (not certified)	0.04 mg/L		
		Pb (not certified)	0.088 mg/L		
		Se (not certified)	0.049 mg/L		
		Zn (not certified)	0.64 mg/L		
Urine Normal CRM	NIST-SRM-2670	Al (not certified)	0.18 mg/L		
		As (not certified)	0.015 mg/L		
		Cd (not certified)	0.0004 mg/L		
		Cr (not certified)	0.013 mg/L		
		Cu	0.13 mg/L	15	a
		Hg (not certified)	0.002 mg/L		
		Mn (not certified)	0.03 mg/L		
		Ni (not certified)	0.07 mg/L		
		Pb (not certified)	0.01 mg/L		
		Se	0.03 mg/L	27	a
Urine Spiked CRM	NIST-SRM-2670	Al (not certified)	0.18 mg/L		
		As	0.48 mg/L	21	a
		Cd	0.088 mg/L	3.4	a
		Cr	0.085 mg/L	7.1	a
		Cu	0.37 mg/L	8.1	a
		Hg	0.105 mg/L	7.6	a
		Mn (not certified)	0.33 mg/L		
		Ni (not certified)	0.3 mg/L		
		Pb	0.109 mg/L	3.7	a
		Se	0.46 mg/L	6.5	a

Suppliers

BCR	Community Bureau of Reference (BCR), CEC, Brussels, Belgium
IAEA	International Atomic Energy Agency, Vienna, Austria
KL	Kaulson Laboratories Inc., West Caldwell, NJ, USA
NIST	Standard Reference Materials, Gaithersburg, MD, USA
NYCO	Nycomed Pharma, Diagnostica, Oslo, Norway
University of Gent	Prof. Dr. J. Versieck, University Hospital, Gent, Belgium

Type of error

a	95% confidence interval for the mean
a*	non-symmetrical form of confidence interval or a small modification from the usual definition
b	± 2 standard deviations of the mean
0	other

Conversion factors from gram to molar units

As	1µg = 13.35 nmol	Cd	1µg = 8.90 nmol	Cr	1µg = 19.23 nmol
Co	1µg = 16.97 nmol	Cu	1mg = 15.74 µmol	Pb	1µg = 4.83 nmol
Mn	1µg = 18.20 nmol	Hg	1µg = 4.99 nmol	Ni	1µg = 17.03 nmol
Se	1mg = 12.66 µmol	Zn	1mg = 15.30µmol		

in the journal Science of the Total Environment in 1993 - 1995 [21 - 24].

The order of magnitude of the trace element concentrations is given in the element specific guidelines.

E. ELEMENT SPECIFIC GUIDELINES

Additional element specific guidelines are given for aluminium, arsenic, cadmium, chromium, cobalt, copper, lead, lithium, manganese, mercury, nickel, selenium and zinc[5,25]

1. Aluminium

Indications for determination and matrices

Although there is some interest in measuring aluminium concentrations in body fluids to assess environmental exposures, the lack of established health effects of such exposures and poor baseline data on expected concentrations together make this a rather unscientific pursuit at present. There is a need for much better reference data on aluminium levels in serum and urine of healthy individuals. In contrast, chronic renal failure and renal dialysis cause marked retention of aluminium with well documented consequences - above certain levels the risk of neurotoxicosis ("dialysis encephalopathy") and bone disease (osteomalacia) increases. For this reason, aluminium is measured clinically and there are well developed interlaboratory comparison and quality assurance programs in place for the clinical analyst. These programs are generally confined to serum aluminium, the indicator of choice for dialysis-related aluminium retention. Both serum and urinary Al are suitable as biological monitoring indices for assessing recent occupational exposure to respirable aluminium compounds.

Precautions and pre-analytical sources of variation

Aluminium is used extensively in metallurgy, packaging, and fabrication of all forms of transport vehicles. Aluminium oxide is widely used as an abrasive and aluminium sulphate is a flocculent important in municipal water treatment protocols. In addition to industries involved in the production or use of these various forms of aluminium, electrolytic production of aluminium itself is associated with increased concentrations of the metal in blood and urine [26].

As aluminium comprises approximately 8% of the earth's crust, environmental exposure is ubiquitous.

Aluminium occurs in most foods and is especially high in some such as tea. It may be added to foods by leaching from cookware or storage containers when the contents are acidic. Aluminium is also a component of many food additives and is added to drinking water during water treatment. Adult Canadians have been estimated to ingest means of 9 - 14 mg Al/day in food and 160 μg Al/day in drinking water [27]. Absorption can be significantly increased by co-ingestion of citric acid [28]. Aluminium is present in buffer-coated pharmaceuticals and aluminium salts are used as phosphate binders to control hyperphosphatemia.

There is little information on age and sex effects on serum or urine aluminium in healthy individuals. As noted above, the major medical conditions associated with increased serum aluminium are chronic or end stage renal disease with or without dialysis. Here, impaired excretion, aluminium in dialysis fluid, and aluminium-based phosphate binders all contribute [29].

Because aluminium is the third most abundant element in the earth's crust, potential contaminants are ubiquitous, and this has perhaps presented the major impediment to establishing reference levels in non-occupationally exposed, healthy individuals. All glass- and plasticware must be thoroughly washed with acid or EDTA solutions and then checked for their contributions of aluminium to the sample. Avoidance of any dust on the sample is essential at all stages. In the analytical laboratory this requires sample preparation in a Class 100 hood and limited access to the laboratory to ensure as little air movement as possible. Samples should be collected in a similar environment, and for venipuncture talc-free gloves must be worn. Water and reagent acids are other likely sources of contamination. Savory et al. [29] have reviewed studies assessing contamination during blood collection. Attainment of blank values at the sub-μg/L range requires extremely careful attention to those details. Specimens from the dialysis clinic are frequently reported only above a cutoff value, typically around 5 μg/L.

Al is also a common constituent of laboratory ware and ultra-pure acids. Data for Al in glass, polythene, polypropylene, and for acids are described in the literature [30].

Analytical methods

The method of choice for measuring aluminium in body fluids is GFAAS and Zeeman background correction is very helpful. Analytical problems have been reviewed [29, 31, 32]. They are best overcome by minimal processing, preparation of standard curves in a matched matrix (e.g. pooled serum), and use of pyrolitically coated graphite tubes with or without a L'vov or stabilized temperature platform. Protein precipitation (as originally described for nickel by Sunderman Jr et al.[32]is very useful for serum aluminium analysis and dilution is adequate for urine.

Analytical requirements for Al measurement differ according to the objective pursued and the matrix studied. In serum (avoid plasma as anti-coagulants contain Al) three distinct concentration ranges of particular interest occur: 1. Al concentrations < 10 μg/L, if not < 1 μg/L: these measurements are only accessible to a few laboratories, since a sensitivity of 0.1 μg/L and very low blanks are indispensable; 2. patients with renal insufficiency moderately exposed to Al: 50 - 200 μg/L serum; 3. patients exposed to Al showing clinical signs of intoxication: > 300 μg/L.

Expected concentrations

Aluminium is ubiquitous in our environment, but very low solubility products of aluminium oxides and hydroxides render their bioavailability to man very low [33]. Nevertheless, mean concentrations of serum aluminium in healthy individuals appear to be about 1.3 - 1.6 μg/L [31,36], may range up to 10 μg/L [21], and may therefor be an order of magnitude higher than some of the transition elements. Urinary levels may be in the upper part of this concentration range [21,31]. Values are much higher in dialysis patients. Whereas values of 100 μg/L are well tolerated by some patients, others develop symptoms that may be attributable to Al at levels as low as 30 μg/L; the "warning level" is generally 60 μg/L [37]. Delves et al. point out the importance of speciation in aluminium toxicity [38]; the Al bound to low molecular weight components (5 -10 kDa) found in dialysis patients is probably

more important than total serum aluminium. With short term occupational exposure at a TWA (Time Weighted Average) of 5 mg/m^3 urinary values of about 200 µg/g creatinine are expected. The DFG (Deutsche Forschungsgemeinschaft) proposes a BAT(Biological Tolerance Value) of 200 µg/L in urine.

2. Arsenic

Indications for determination and matrices

The inorganic forms of As (As^{III} and $As^{V)}$ are far more toxic than the organic ones. Arsenic taken in through sea-food consumption is predominantly arsenobetaine and arsenocholine with little or no toxic effect.

In industry, workers are usually exposed to inorganic arsenic species. For biomonitoring it is necessary to perform the analysis of As in the biological fluids in a species-specific way. The analysis of total As may give rise to misleading results as the evaluation is dependent on the level and the species of dietary arsenic. Elevated As concentrations may be due to the consumption of seafood containing high amounts of the non-toxic compounds arsenobetaine and arsenocholine. If possible consumption of seafood should be avoided for at least 3 days before sampling for As-measurements.

Arsenic levels in blood and urine have a short biological half-life and reflect recent exposure, and in case of As in blood only that of the last few hours. Hence it is significant to know the time for the onset of exposure, its duration and the sampling time. This is very important in biological monitoring.

Precautions and pre-analytical sources of variation

The collection of blood and urine for As measurements appears also to be easily prone to contamination from As in reagents, dust and laboratory ware[39].
Arsenic in blood is expected to be stable at -20°C.
No preservatives are added to urine, but the bottle should be completely filled to prevent oxidation of As^{III} to As^{V} from the air in the bottle. The samples are kept refrigerated, and are stable at -20°C for more than 6 months.

Analytical methods

The different As-species are separated by ion-pair liquid chromatography, and after transformation measured by hydride-generation atomic absorption spectrometry equipped by an electrodeless discharge lamp (EDL) for As. The method is applicable to both serum and urine samples[40-42].

Expected concentrations

The serum and packed cell As concentrations depend on the level of dietary intake of As, more particularly on the amount of seafood intake and on the arsenic content of the water.In seafood As is mainly present as organoarsenicals with negligible toxicity. Values in healthy individuals vary between 1 and 5 µg/L serum. Patients with renal insufficiency on conservative treatment and on chronic dialysis are reported to have markedly increased As levels in serum and packed cells [43-44]. In case of occupational exposure to inorganic arsenic, the levels of the toxicologically important arsenic species As^{III}, As^{V}, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) will be increased. Therefore speciation of the arsenic species is recommendable to interpret the results.

Biological monitoring of arsenic in workers exposed to inorganic arsenic is carried out by measuring the total amount of arsenic present in urine collected at the end of the shift or at the beginning of the shift. Speciation of the As-species is mandatory for a correct interpretation of the data. Arsenic concentration in urine mainly reflects recent exposure.

In Europe the As concentration in urine of non-exposed individuals is about 10 μg/L, whereas in Japan it is 50 μg/L or higher [25,45].

3. Cadmium

Indications for determination and matrices

It is unlikely that cadmium is essential for higher animals; rather, it is very toxic. Reports of weak carcinogenic activity are inconclusive [46]. Acute poisoning by accidental ingestion or, in the past, by inhalation of fumes with a high cadmium content, is rare. Chronic exposure, on the other hand, is an important occupational problem with nephrotoxicity the primary manifestation. Therefore, measurement of cadmium in body fluids is widely used for exposure monitoring, and is generally required at least annually in the higher risk settings. Because cadmium accumulates in the erythrocyte, whole blood cadmium should be measured for this purpose and is indicative of both body burden and recent exposure [25]. Urine cadmium is also used for monitoring and assessment of body burden. Plasma or serum cadmium concentrations are not useful for this purpose and are not well studied. Environmental exposure to cadmium is also variable (see below), and so there is interest in setting reference levels of cadmium in body fluids in geographically and ethnically distinct groups.

Precautions and pre-analytical sources of variation

Based on numbers of workers employed and atmospheric concentrations of cadmium that can be attained, those at high risk of occupational cadmium exposure include smelter and refinery workers, alloy and battery makers, pigment and plastic workers, plate workers and welders. Many other occupations present a lesser problem. In some of these, however, the risk is high although the numbers affected may be relatively small (e.g. pewter manufacturing).

For non-smokers, food is the major source of cadmium exposure, with typical intakes of 10 - 60 μg/day in some regions but much higher in others due to diet and the environmental distribution of cadmium [47]. For instance, in Japan, daily intakes are from 60 μg to several hundred [48]. Certain foods (e.g. organ meats, some shellfish) are especially high in cadmium. Smoking is an important source of cadmium. In those not occupationally exposed, it has been estimated that blood cadmium increases 1.6 % per cigarette smoked per day [47]. Thus, smokers have significantly higher concentrations of blood cadmium than non-smokers - typically double in age-matched cohorts.

Because cadmium accumulates in the soft tissues (chiefly liver) bound to metallothionein, and has a long biological half life (10 - 30 yrs) in man, cadmium organ content increases with age. This is not necessarily reflected in increased blood concentrations, which better indicate recent exposure. Nevertheless, cadmium does leave soft tissue stores over the long term and blood and urine concentrations do increase with age, although urine cadmium decreases again in the elderly. Cadmium is initially sequestered in the liver as cadmium metallothionein, and its transport to kidney is a later event heralding nephrotoxic manifestations. When body stores become saturated, newly acquired cadmium will spin into the urine. Prior to that time, urine cadmium is a better indication of body burden. When renal damage begins to occur, excretion of cadmium can rise markedly. Women may have higher blood concentrations of cadmium than men because of higher absorption in the gut, perhaps secondary to lower iron stores [47]. Therefore, it is especially critical when assessing reference values for cadmium in non-occupationally exposed individuals to have information on age, sex, diet, occupation, place of residence, and smoking habits of the reference population.

The use of cadmium in pigments and as a softener in plastics necessitates avoidance of sample contact with coloured stoppers and certain plastics during collection and processing. Glass should also be avoided. Plastic syringes and test tubes should be cleaned and tested for their ability to release cadmium. No special needle is required: stainless steel is adequate. Talc-free gloves should be worn by the venipuncturist.

Analytical methods

Graphite furnace- (ET-) AAS is presently the method of choice for blood and urine cadmium. Blood or urine is mixed with concentrated nitric acid, centrifuged, and the supernatant can be analyzed directly [49, 50], preferably with Zeeman correction. ICP-MS works well for cadmium, with several isotopes available for study and excellent detection limits in multielement protocols. The interest in portable electrochemical instruments for blood lead analysis is driving the development of voltammetric techniques that are readily applicable to blood cadmium, and may eventually become the method of choice [51].

Expected concentrations

Like a number of other trace elements, reported levels of cadmium in body fluids were probably too high in many earlier studies due to poor analytical sensitivity or lack of control of contamination. As more reliable data become available, however, it is beginning to appear that differences in blood cadmium concentrations in the range 0.1 - 2 μg/L do occur in different reference populations and arise from differences in environmental exposure as well as other biological factors, rather than from analytical error. For example, Friberg and Vahter [52] found (geometric) mean values of blood cadmium in healthy non-smokers to be 0.19 μg/L in Sweden, 0.5 - 0.6 μg/L in the USA and China, and 1.06 μg/L in Japan. The higher concentrations in Japan are consistent with numerous other reports, and reflect the high consumption of local rice irrigated with water having a higher cadmium content. Concentrations in urine of similar populations are generally < 1 μg/L [47]. The ACGIH has adopted a BEI of 5 μg/L in blood. The corresponding urine concentration is about 3 μg/g creatine. According to OSHA, 5 μg/g creatinine warrants assessment of operational practices.

4. Chromium

Indications for determination and matrices

Toxicity of Cr is mainly due to Cr^{VI} that can be absorbed by the respiratory tract and also to a certain extent by the intact skin [25].

In serum Cr occurs as Cr^{III} and is bound to serum proteins, especially transferrin and albumin. Cr^{VI} absorbed during inhalation rapidly penetrates the erythrocyte membrane, is then reduced to Cr^{III} and binds predominantly to haemoglobin. In urine chromium occurs as Cr^{III}. Cr-compounds are also easily absorbed through the skin. Cr in urine measurements appear the most suitable indicator for biological monitoring of chromium exposure.

Chromium from food and beverages is poorly absorbed , and is excreted in the urine.

Precautions and pre-analytical sources of variation

Analytical requirements for the measurement of Cr in blood and urine are very stringent in order to ensure a reliable sample and hence accurate data.
A stainless needle cannot be used. A propene i.v. cannula is compulsory (see general guidelines). A needle siliconized on the inside could be tested for its reliability. In all cases the first 20 ml of blood drawn through the needle cannot be used for Cr-analyses. Anti-coagulants should be checked on their Cr-content. Acid-washed plastic tubes are required for blood collection. Unwashed commercially available tubes will invariably be found to contribute too high values for Cr in the blank. The Cr-content of sweat is about ten times higher than that of serum [7]. Therefore contamination from the skin has to be avoided.

Patients on haemodialysis and on peritoneal dialysis have markedly increased serum Cr concentrations [53-54].

Urine should be collected in acid-washed vessels. Acidification of the samples with nitric acid or acetic acid is recommended.

Analytical methods

Graphite furnace atomic absorption with background correction is usually used for Cr measurements in serum [55] and urine [56-59]. Zeeman background correction is very appropriate. The preferred method for the measurement of Cr in erythrocytes is also graphite furnace atomic absorption spectrometry [60].

Expected concentrations

Researchers who analyzed Cr in serum obtained from blood samples taken in meticulously controlled circumstances as far as avoidance of contaminations is concerned, agree on a Cr level of 0.1 - 0.2 μg/L [5]. The concentration of Cr in urine of non-exposed individuals lies ≤ 1 μg/L or 5 μg/g creatinine. The end of shift urinary Cr level of workers occupationally exposed should not exceed 30 μg/g creatinine after several weeks of exposure. The increase during the shift should not exceed 10 μg/g creatinine [25].

5. Cobalt

Indications for determination and matrices

Cobalt is essential for humans in the form of vitamin B_{12} and in the absence of impaired B_{12} absorption deficiency does not occur. The absorption of cobalt compounds after inhalation or ingestion is dependent on solubility; soluble forms are readily absorbed and excreted in the urine. Apart from biological curiosity, the only indication for cobalt measurement in the human population is for monitoring of occupational exposure, and for this purpose urine cobalt suffices and is reliable after exposure to soluble, but not insoluble, cobalt compounds [61]. Cobalt and its compounds are classified by IARC in Group IIB - possibly carcinogenic to humans [62]. Formerly ingestion of large amounts of cobalt salts as an additive in beer or in the treatment of anaemia resulted in fatal cardiomyopathies [63]. Today the major health concerns are for cobalt sensitization and occupational asthma from exposure to cobalt compounds [64, 65], and interstitial lung disease from co-exposure to cobalt and other metal carbide dusts [65, 66].

Precautions and pre-analytical sources of variation

Obvious exposures to cobalt metal and fumes occur in the metal production and refining processes and the production of cobalt compounds in the chemical industry. Cobalt is used as a matrix or binding agent in the fabrication of cemented metal carbides ('hard metals') such as tungsten carbide and their widespread use in drilling and machining is a source of inhalation of cobalt, usually mixed with metal carbide dusts, chiefly tungsten, titanium and tantalum. Dermal exposures to cobalt salts, pigments, and other organic cobalt compounds occur in the rubber industry and tire manufacture, and in the manufacture and use of paints and varnishes, pottery decoration, and inks for offset printing. Soluble and insoluble cobalt compounds in cement are a source of dermal exposure in the construction industry. Diamond polishing with microdiamonds cemented in high purity cobalt powder results in inhalation of cobalt-containing dusts. Additional exposures are from the use of magnetic alloys (chiefly in the telecommunications and electronics industries), the so-called super alloys (used for jet and gas turbine engines), and high strength steels. Dental technicians are also exposed to cobalt alloys.

It is mandatory to collect blood with devices that give no measurable Co-blank. Stainless steel needles are excluded for this type of measurements. All containers must be acid washed. Urine samples are acidified with nitric acid and stored at 4°C for one week or -20°C for longer periods.
Co in blood is stable for many years at - 80°C and possibly at -20°C. The availability of a class 100 work area is strongly recommended.
Non-occupational exposure to cobalt arises from surgical implants and dental prostheses, and contact with metallic objects such as jewellery. Individual intake from food is somewhat variable, but typically 10 - 100 μg/day [62, 63]. An exception is those taking vitamin supplements containing B_{12}. A slight increase in mean urine cobalt in patients with cobalt-alloy knee and hip prostheses was reported [67].

There is little or no information on biological variability in cobalt concentrations in body fluids arising from these natural exposures. Oral administration of $CoCl_2$ to men and women gave rise to higher urinary cobalt in the women [68]. Because iron deficiency increases cobalt absorption in the gut [69], this might be a reflection of body iron stores.

Sample contamination is a major problem in analysing body fluids of non-occupationally exposed people for cobalt. The precautions described for nickel apply - careful washing of the skin, avoidance of steel needles, thorough acid washing of all materials contacting the sample, and sample processing in a clean air environment.
Co in urine has a short biological half-life reflecting recent exposure within the last few hours. In addition, there are several more half-lives ranging from a few months up to several years, due to the body-burden of Co. Hence it is of major importance to know the exact time lapse between the onset of exposure and sampling. To evaluate recent exposure, the best protocol consists in collecting several samples at short intervals along the working day.
In case of spot urine samples, be aware that the first urine, which rested in the bladder during the night, has a high density. Therefore the first void could give aberrant results.

Analytical methods

Previously chelate extraction or pre-concentration were necessary before measuring cobalt by ET-AAS. Today direct analysis by Zeeman-corrected ET-AAS is the method of choice for routine measurement of cobalt in body fluids, and is the basis of recent publications found useful for establishing tentative reference values (Christensen and Apostoli, to be published). Urine may be analyzed after acidification and dilution, serum after deproteinization, and whole blood after digestion. Other methods useful at the expected reference levels include pulse and stripping voltammetry [70, 71] and neutron activation analysis [75].

Expected concentrations

The reference values of cobalt in the body fluids of healthy individuals are not known with certainty. From a recent critical analysis of the literature it appears that values of urine cobalt are in the range 0.1 - 1 μg/L with values in serum and blood at the lower end of this range [4](Christensen and Apostoli, to be published). At a TWA exposure to soluble cobalt compounds of 0.05 mg/m^3 a urine cobalt concentration of 30 - 40 μg/L is found consistently, while the biological exposure index corresponding to a TLV of 0.02 mg/m^3 is 15 μg/L [61]. The corresponding concentrations in serum are more than an order of magnitude smaller.

6. Copper

Indication for determination and matrices

Cu metabolism is homeostatically well controlled. The levels are influenced by physiological (such as in pregnancy) or pathological conditions (e.g. in some liver diseases).

Precautions and pre-analytical sources of variation

There seem to be no major methodological problems, neither evident contamination hazards associated with the measurement of copper in biological fluids.
A well documented medical history could be very helpful for the interpretation of abnormal results.
No special analytical precautions are to be considered for the measurement of Cu in biological fluids.

Analytical methods

Cu can be easily measured by flame and graphite furnace atomic absorption spectrometry, although there is no special advantage in using the latter[76].

Expected concentrations

The copper concentration in serum or plasma of healthy individuals covers a range from 0.8 to 1.4 mg/L. In urine the mean urinary concentration ranges from 15 - 36 µg/24 h [4, 5].

7. Lead

Indications for determination and matrices

Lead is toxic for humans even at low levels. Chronic effects on heme synthesis are reported on inhibition of the enzyme δ-aminolevulinic acid dehydratase in blood, reduction of concentrations of porphyrins in blood (especially zinc protoporphyrin), δ-aminolevulinic acid in urine, coproporhyrin in urine, and, in increased dose, haemoglobin itself [77,78]. Effects on blood pressure are reported as well [79]. Effects on the kidneys are reported as enhancement of the tubular enzyme N-acetyl-ß-D-glucoseaminidase in urine [80]. Numerous cases are documented in which heavy lead exposure caused encephalopathy in children and adults. In recent years also concern has been rising about cognitive deficits in children on dose levels of lead in blood (B-Pb < 400 µg/L) [78].

Lead in urine reflects the amount of Pb recently absorbed. Blood lead represents the concentration of Pb in soft tissue and of recent exposure. Therefore, B-Pb is used as a biological monitor measure, both in occupational and environmental health studies and screening programs.

Precautions and pre-analytical sources of variation

Blood Pb levels are usually measured from analysis of venous blood.
In case of finger prick blood - a procedure that is not recommended - the extremes of the finger have to be carefully cleaned, twice with double distilled water and twice with analytical grade ethanol (the latter without cotton, just rinse) and allow to dry by evaporation.

Sampling of blood for Pb measurements can be done using disposable sampling devices and containers.

The anti-coagulant must be tested on its Pb-content.
Blood can be stored in the refrigerator for a period up to 3 weeks.If longer storage is needed, the samples have to deep frozen at -20°C.

Extreme care is needed during the preparation of serum, as the concentration of Pb in serum is 10% of the concentration in whole blood, with the remaining 90% located in the packed cells. Even marginal haemolysis during blood collection and subsequent serum separation will be responsible for a much elevated (factor 2 or more) Pb concentration in serum. Therefore it is necessary to estimate for all serum samples the degree of haemolysis by measuring the haemoglobin concentration.

The Pb blank caused by chemicals and materials must be sufficiently below the Pb concentration in serum in order to avoid misleading results.

Analytical methods

Graphite furnace atomic absorption spectrometry, if possible with Zeeman background correction, is the most commonly used method for the measurement of Pb in blood and urine [81,82].

Triton X-100 is added to whole blood to reduce the viscosity of the sample.

Although lead in blood is the element determined most widely, problems are still manifest. Quality assurance including internal and external quality control of lead in blood determinations should be therefore an integrated part of the determination. Problems arising with the determination are related to contamination of the blood appearing from vena puncture until the final determination, and the GF-AAS measurement. Evidently endogenous lead in blood behaves differently from the standards,

both aqueous and standard addition. Therefore a matrix modifier is absolutely necessary to overcome these problems, e.g. $Mg(NO_3)_2$ or $NH_4H_2PO_4$. Even then matrix-matched standards or standard addition will be necessary.

Expected concentrations

The lead in blood concentration of individuals in the general population is dependent of the year of sampling [81] and may be related to the use of leaded or unleaded gasoline in a country or region. Thus, it is very important that both the years of sampling and the region are mentioned in a study.

Examples of reliable (geometric) mean values are: 1983, Sweden 72 μg/L - Mexico 225 μg/L [83], and in 1992 Sweden 29 μg/L - China 73 μg/L [84].

Threshold levels for use in occupational health are: ACGIH BEI level 500 μg/L and German BAT value 700 μG/L for men and 300 μg/L for women aged below 45 years.

8. Lithium

Indication for determination and matrices

The measurement of Li in serum is important for monitoring patients suffering from manic-depressive psychosis, treated with lithium carbonate. A well documented medical history is necessary for the evaluation of the Li results.

Precautions and pre-analytical sources of variation

Usual clinical practise suffices for the collection and storage of samples for Li measurements for clinical purposes.

Analytical methods

Lithium can be measured directly in serum and whole blood by graphite furnace atomic absorption spectrometry [85]. Another method which is commonly used is measurement of Li in serum with an ion-selective electrode [86]. There seem to be no major problem arising for the measurement of the therapeutic doses of Li in serum and whole blood. Other methods are flame atomic absorption, emission spectrometry and ICP-MS [87].

Expected concentrations

The Li level in serum or plasma of healthy individuals is reported to be at the 1 μg/L level [88]. In case of patients on Li-therapy, the concentration is markedly increased and depending on the dose-schedule may increase to the mg/L level [89].

9. Manganese

Indication for determination and matrices

In industry workers absorb Mn mainly through the lungs [25]. Excretion occurs mainly trough the bile. Although the excretion in urine is low, the determination of Mn in urine is used to estimate recent exposure.

Precautions and pre-analytical sources of variation

Manganese is a most difficult element to measure reliably in human samples because of multiple possibilities of contamination.

Practically all Mn in blood is linked to the packed cells. Therefore extreme care is needed during the separation of the serum and packed cells. Even a marginal haemolysis during blood collection and subsequent serum separation will elevate the Mn concentrations and render the data obsolete.

The Mn concentration in urine is also very low, so that very strict rules must be respected during the collection and the handling of the samples.

During preparation and analysis Mn contamination by dust particles from the laboratory air and the surface of the recipients should be kept in mind. Contamination of the samples should be excluded by taking appropriate measures, such as working in class 100 circumstances.

For reliable sampling, the use of a teflon canula is recommended, since stainless steel needles have been shown to leach Mn into the blood serum [4, 5].

Sampling syringe and storage vial must be controlled prior to use because contamination with Mn is possible. If no special precautions have been taken by the manufacturer then these items cannot be used as such. If anticoagulants are used testing for contamination is compulsory.

Cleaning of the skin is compulsory as Mn is excreted by sweat [7]. Tap water can contain high amounts of Mn (>1 mg/L).

No losses are expected when the sample is stored at -20°C, or freeze dried.

Analytical methods

Graphite furnace atomic absorption, preferably with Zeeman background correction, is suitable for the direct measurement of Mn in whole blood, serum and urine [89, 90].

Expected concentrations

The Mn concentration in serum of healthy individuals amounts to 0.5 µg/L. In packed cells the level is about 15 µg/kg and in whole blood about 8 µg/l [4, 5, 76]. In urine of non-exposed individuals the Mn level amounts to the 1 µg/L.

10. Mercury

Indication for determination and matrices

The main routes of uptake of mercury are from inhalation and gastrointestinal absorption. The degree of toxicity is dependent on its chemical form. Inhaled metallic Hg vapours have a very high retention rate in the body. On the contrary gastrointestinal absorption of metallic mercury is negligible. The absorption of methylmercury compounds from food may cause serious health hazards.

Hg in blood is measured to estimate exposure. Similarly Hg in urine measurements are used for biological monitoring. The sampling time during the day is very important for the interpretation of the results [91, 92].

Precautions and pre-analytical sources of variation

It is important to avoid contamination of the biological fluids with mercury. Blood contamination by anticoagulants has been reported and so the blank value has to be measured. Cleaning of the sample vessels or at least checking of their possible contribution to the actual mercury levels is compulsory.

Blood samples can be stored for a few weeks in the refrigerator, but for longer periods storage in the deep freezer at -20°C or below is recommended.

To avoid Hg absorption by the container wall, the urine samples have to be acidified with nitric acid or acetic acid. They can be stored in the refrigerator for many weeks, or deep-frozen at -20°C. It is important to avoid bacterial growth as this may reduce some mercury to volatile elemental mercury [93].

Analytical methods

Cold vapour atomic absorption and atomic fluorescence are both very sensitive and reliable techniques for Hg measurement in blood, serum and urine. The Hg-species are converted by reducing agents (e.g. $NaBH_4$) to elemental mercury and released as vapour which is either directly pumped through the cell of the atomic absorption spectrophotometer or analyzed after amalgamation and enrichment on Au e.g. sand with a Au layer, or Au/Pt gauze) [93].

Expected concentrations

The Hg concentration in serum of healthy individuals amounts to 0.5 μg/L. In packed cells the level is about 5 μg/kg [76]. In urine of non-exposed individuals the Hg level lies at the 1 - 10 μg/L [76].

11. Nickel

Indications for determination and matrices

The essentiality of nickel in humans remains questionable. Soluble nickel compounds are readily absorbed in the gut and following inhalation, and excreted by the kidney with very low acute toxicity [73, 74]. Nevertheless, nickel is immunotoxic and induces skin sensitization that effects about 10 % of women and a smaller number of men [94, 62]. The carcinogenicity of nickel has been extensively reviewed in Committee [96, 97] and IARC has concluded that nickel compounds - but non metallic nickel - are carcinogenic to humans (Class I). Therefore, occupational exposure is monitored, urine and in some circumstances serum being appropriate samples. Reference values in body fluids of non-occupationally exposed individuals have not been established, and analysis of urine, serum, plasma, and whole blood for total nickel content remains of academic interest.

Precautions and pre-analytical sources of variation

Occupational exposures to nickel are widespread and occur in mining and refining of nickel ores, production of alloys including stainless steel, electroplating with nickel, and stainless steel welding. Magnetic nickel alloys are used in the electronics industry. Manufacture of nickel-cadmium batteries gives rise to exposure to both metals. Additional sources include the chemical, pigment (e.g. $NiTiO_3$) and ceramics industries, catalyst production (e.g. Raney nickel), and making metal objects such as jewellery, coins, and medical prostheses. Waste incineration and burning of fossil fuels also lead to increased inhalation of nickel. Uses of and exposures to nickel compounds have been reviewed thoroughly by IARC [118].
In populations without significant occupational exposure, dietary intake of nickel is the major source of exposure, being at least 100 μg/day [97] and up to nearly 1 mg/day in some vegetarian diets [98]. Urban atmospheres in the United States contain about 25 ng of nickel per m^3 but values of about 150 ng/m^3 have been recorded in polluted areas, particularly where fossil fuels are burned. This also introduces seasonal variation. Urban dwellers are reported to inhale 0.2 - 1.0 μg/day [98, 99]. Nickel in cigarette smoke may increase this value by as much as 4 μg/pack. Non-occupational exposures also arises from handling metal objects such as jewellery and coins, and from implantation of medical prostheses made of nickel-containing alloys.

There are too few reliable studies to comment on differences in nickel concentrations with age or sex. Among the more reliable studies of serum nickel and reported increases, are those stipulating a range from 3 to 7 μg/L in patients with renal failure and impaired renal excretion [100-102], an approximate doubling to 0.6 μg/L in rheumatoid arthritis [7], and a transient increase of about ten-fold following acute myocardial infarction [103].

Contamination presents a major problem that compromises many studies of nickel in body fluids. Because the concentration of nickel is sweat is several times higher than in serum [7] and concentrates further on drying, the skin must be carefully washed before collecting blood. The use of a stainless steel needle is generally thought to preclude analysis for nickel and Teflon cannulae are preferred, although one study has achieved apparently good results with stainless steel needles by discarding the first 3 mL of blood [102]. All materials coming into contact with the sample (syringe, plastic pipette tips, etc.) must be cleaned by an acceptable acid washing procedure [102]. Sample manipulations should be carried out in a Class 100 air environment [104].

Analytical methods

Direct analysis by Zeeman-corrected ET-AAS [32, 105] has largely replaced an earlier need for chelate extraction or pre-concentration [106], and reports found useful for establishing tentative reference values [107] have all used this method. Urine may be analyzed by Zeeman ET-AAS after acidification and dilution, serum after deproteinization, and whole blood after digestion [32, 103]. Other methods useful in the research setting include voltammetry and mass spectrometric techniques including ICP-MS [105].

Expected concentrations

Reported reference values of nickel in body fluids of healthy individuals without occupational exposure have been steadily revised downwards. Based on a review of the recent literature, Templeton et al. [107] have proposed tentative reference values in serum and urine of < 0.3 μg/L and < 3 μg/L, respectively. At a TLV exposure of 0.1 mg Ni/m^3 in air, soluble nickel compounds produce a urinary concentration of about 70 μg Ni/L, while for less soluble forms the corresponding value is about 15 μg/L [108]. The same exposure to soluble nickel compounds has been reported to give a plasma nickel concentration of 7 μg/L [109].

12. Selenium

Indications for determination and matrices

In blood selenium is partly present as selenocysteine in the enzyme glutathione peroxidase (GSH-Px), be it that normally only a very small fraction of total Se occurs as GSH-Px. This explains the general interest of measuring both Se and the glutathione peroxidase activity of the blood samples. Another part of Se in blood is present as selenomethionine [110].

Precautions and pre-analytical sources of variation

In sharp contrast to most trace and ultratrace elements the sampling procedures are essentially free of contamination problems. Standard equipment for sampling of body fluids can be used.

Since selenium in whole blood and in serum/plasma is also associated with a variety of proteins, any protein precipitation during storage should be avoided. Storage of samples should adhere to common practise, short term <5°C, long term < -20°C.

Analytical methods

Se is routinely measured by graphite furnace atomic absorption spectrometry [111]. As Se is subject to losses during the drying and charring steps, matrix modification techniques are recommended. Zeeman background correction is very useful.
Hydride generation atomic absorption spectrometry offers a superior sensitivity for the detection of Se. Complete mineralization of the sample is indispensable to ensure that all Se can be transformed to H_2Se by using $NaBH_4$ as a reductant.

Expected concentrations

The serum or plasma Se concentrations in adults vary between 0.04 and 0.16 mg/L depending on the Se intake from food and beverages [5]. Se is present in packed cells in the same order of magnitude.

The Se concentration in urine is at about 100 µg/L, but is very dependent on the Se-intake [20].

13. Zinc

Indications for determination and matrices

The medical history of the patient should be well documented because of the use of Zn-containing drugs, that are liable to influence the concentration in blood and urine. Zn screenings are widely applied in the clinical world.

Sampling in the morning after an overnight fast is mandatory because of pronounced diurnal changes and a fall of plasma Zn concentrations after meals.

Precautions and pre-analytical sources of variation

As the Zn concentration in packed cells is about 10 times higher than in serum or plasma, it is necessary to ensure that no visible haemolysis occurred in the blood samples. The degree of haemolysis should be investigated. The Zn-blank caused by the anticoagulant has to be checked for each batch.

The serum, packed cells and blood samples can be stored in a refrigerator at 4^0C to 10^0C for several weeks.

The threat of contamination during sample collection and processing is very outspoken [4,5]. Gross contamination problems may occur due to the collection vials, including the stopper. Careful acid washing of all glassware and plastic ware, followed by rinsing in pure water is mandatory. The complete cleaning procedure has to be checked regularly to assess the blank value.

Analytical methods

Flame atomic absorption spectrometry is a very suitable method for the measurement of Zn in biological fluids. Because of its 1000 times superior sensitivity, graphite furnace atomic absorption spectrometry offers the possibility to work with very small samples [112].

Expected concentrations

The serum or plasma Zn concentrations amount to 1 mg/L [5]. Zn packed cells is one order of magnitude higher.

The Zn concentration in urine may vary between 50 - 1 000 µg/day depending on the Zn-intake [20].

F. CONCLUSION

The important conclusion of this work is that every step of the analytical procedure: subject identification and description, presampling, sampling [119], sample manipulation, analytical method, measurement, calibration, evaluation and quality assurance should be scrutinized. The data should be presented as completely as possible, citing the whole range covered, the 95% percentile, test for normality, and mean ± s.d. The aim is harmonization of results worldwide, an endeavour shared by the ICOH (International Commission on Occupational Health) [113] the European Union [22] and the World Health Organization [16].
Finally the work of Alessio et al.may be cited [114, 115].They emphasize the standardization of inclusion/exclusion criteria for subjects forming the reference group, and on the thorough scrutiny of the analytical methodologies.

Appendix 1

Mastersheet for sample collection guidelines

Part A: Sample information

Memo to the physician and the nursing staff: the following gives examples of type of information that may be needed to collect. It is important to reply to as many questions and as exactly as possible, in order to allow an efficient categorization of the results.

Matrix
Sample code
Name of subject
Address (Domicile)
Date of collection
Time of day
Time relation to meals (see e.g. As)
Place
Name of institution
Name of person collecting the samples
Sampling area: institution, home, work, elsewhere,specify

Description of smoking habits of the person collecting the samples may also be important in case of e.g. Cd.

If the samples are collected at work, then this should take place in a suitable, cleaned room. The worker should change clothes before entering the room to reduce the risk of contamination. Smoking is strictly forbidden.

Personal history

Name
1. Sex, Female, Male

2. Date of birth, year/month/day

3. Employment
   Presently employed? yes, no, specify
   Add information on occupational history, if exposure is indicated, and include detailed information on work processes; i.e. welder
   List all jobs for the last 5 years for unexposed persons.
   Unemployed, since when?
   Hidden exposure: occupation of wife/husband

4. Present workplace
   Office, shop floor, classroom, construction site?
   Exposure to dust, odours in workplace, to chemicals? yes,no
   if yes, describe

5. Special work place environment
   Detailed information should be obtained from the company, as many workers will either be ignorant about the exposures or will not indicate all relevant exposures.
   if yes, indicate
   e.g. radiation, organic solvents, detergents, solid aerosols, fumes or vapours, tobacco smoke

6. Smoking habits
   No, never;
   Yes, formerly; stopped, when?
   Yes, presently, record total years
   if yes: type, cigarettes, cigars, pipes, average number per day
   Do you inhale smoke? yes, no
   Passive smoking, at home or at work, yes, no
   Previous smoking: how many years?

7. Avocations
   (including hobbies, commitments outside work, recreational activities, exercise)
   Describe, including tools and products if relevant
   Home maintenance (within the last 4 weeks)

8. Dietary habits
   Vegetarian, yes, no
   Consumption of fish, crustaceans, shellfish, meat, vegetables, canned food (indicate frequency/week of each)
   Supplements with vitamins or minerals (give brand name)
   Drinking water supply
   Daily use of tea, coffee, water, other non-alcoholic beverages, which? volume
   Alcohol consumption per week, volume
   Type of liquor (beer, wine,..), volume
   Eating and drinking at the work stations, including hobbies

Besides a statement about the general habits, a detailed description of the major ingredients consumed during the last 3 days is mandatory.

9. Area of residence, during the last 5 years, and periods

   Urban, suburban, city center (size of city), industrial (heavy, light), farm, rural, seaside

   Special sources of pollution, specify
   (this is not generally known to the public, instead detailed information may be obtained from the local environmental authorities)
   Air supply (circulated/conditioned)
   Heating (none, electric, fossil fuel, other)
   Type of water pipes (e.g. lead pipes)

10. Health status

   This section should be completed by a physician

   Liver tests
   Serum albumin; substance concentration
   Serum transferrin; substance concentration
   Medical history: diagnosed illness, chronic illness
   Surgical history, incl. implants, prostheses and amalgams
   Obstetrical history
   Is the subject presently under treatment for any major medical problem?
   Medication, specify past prescription drugs and time and current medication
   Oral contraceptive or other hormonal treatments in past year, specify
   Chronic treatment
   Non-prescription drugs in past year

All medications taken in past 48 h
Hypertension
More detailed clinical inquiries may be requested for certain studies

11. Element specific questions

Is the subject aware of a particular source of the element in his surroundings, diet, cooking utensils, tools (e.g. stainless steel,..)?

Specific sources known by the investigator: quality of the air, of the water, food (specify), others

Part B: Sample collection

1. Blood

The laboratory staff has to be informed about the health hazards involved in manipulating blood samples (all patients are potential carriers of e.g. HIV, hepatitis,....)

1.1. Equipment and cleaning procedures

Cleaned collection tubes and stoppers
Specify brand of collection tube
Specify material of tube and stopper
The whole set (tube + stopper) has to be tested according to one of the procedures outlined below, also if they are purchased as "so-called" free from trace element contaminants. The amounts leached out of the materials have to be measured and reported. Specify the limit of detection (LOD= 3 σ of background [116]).
The type of blank samples (certified blood, certified serum or mild nitric acid solution) will depend on the facilities of the laboratory (see below).
In case the tubes + stoppers are cleaned in the house, a similar procedure is mandatory.

Cleanliness test
In order to test the possible leaching of trace elements out of the container wall and out of the stopper, a blood sample, containing a low and known concentration of the elements of interest, should be stored in fourfold at 4° C for 5 days prior to the analysis by a reliable method with sufficiently low detection limit and analytical blank. If the analyses yield a value which is within the typical concentration range, chances are that the degree of contamination is negligible. This supposes that the analytical procedures are under control. This test then covers the quality of the blood collection as a whole (needle, tube, and further on the quality of the blood, serum, plasma, packed cells and erythrocytes).
The alternative way of testing would be a mild nitric acid rinsing (0.03 mol/L followed by the analysis of the leaching solution.

Polyvinyl or latex gloves, free of talc (powder-free gloves)

The needle
Propene or teflon intravenous cannula, mounted on a trocar or siliconized needle or stainless steel needle for a limited number of elements.
Be aware that siliconized needles are often only covered on the outside, for smoother introduction

Test tube racks, polyethylene covered

Air-tight plastic transport container

Anti-coagulant
Specify brand.
In case plasma has to be collected, the a-priori analysis of the anti-coagulant for the trace elements under investigation is a basic requisite. The same remark applies to the tubes and pipetting devices used to prepare the plasma.

1.2. Blood collection and separation of serum and packed cells

The pressure of stasis should be low
Blood should be drawn with the subject supine

Cleaning of the skin of the antecubital fossa of the arm with double distilled water and analytical grade ethanol (the latter without cotton, just a rinse), and allow to dry by evaporation

A tourniquet is applied lightly if necessary, the physician / nurse puts on gloves, venipuncture is done with the needle. In case the intranule is used, the stylus of the catheter is withdrawn whereas the propene or teflon catheter is left in the blood vessel. When sampling adults, the first 10 mL of blood are withdrawn and preserved for e.g. clinical laboratory tests. These 10 mL may be contaminated by contact with the trocar and are also needed to rinse the inner tubing of the cannula. This blood should not be used for trace element analyses. In monitoring programmes involving children, 5 mL may be more realistic. This preliminary rinsing of the trocar and the cannula with blood is compulsory in case of Co, Cr, Mn and Ni in serum and packed cells, and is highly recommended for As in blood and its derivatives and for Cd and Pb in serum. A smaller volume may be sufficient provided this is carefully checked.

The samples are collected in the cleaned collection vials and transported to the laboratory. The blood is allowed to clot spontaneously (at room temperature: 20 min in glass; 1 h in plastic), unless an anti-coagulant was added.

Serum is separated by centrifugation at 1 500 g per minute for 20 min. The serum is decanted into a new clean tube. A second centrifugation may or may not be necessary at 1 500 g for 15 min to remove remaining blood cells, then decanted and stored in clean vials, stoppered and stored at < 5°C. In certain circumstances it may be easier to pipette from one tube to another in which case the transfer device must be thoroughly cleaned and acid washed.

Haemolyzed samples cannot be considered for analysis of their trace element content. Theoretically all samples will be haemolyzed to some extent. Defined criteria may be needed, e.g. visible haemolysis or spectrophotometrical measurement of haemoglobin, which will show if the degree of haemolysis is below a critical value. A realistic approach would be to tolerate haemolysis to the extent that the cross-contamination is lower than the analytical uncertainty on the trace element concentration. The degree of haemolysis can be evaluated by measuring the Zn and (or) Fe content in serum or plasma.

1.3. Storage

Frozen at -20°C or less (in plastic tubes), or in the refrigerator at < 5°C. Warning: especially full tubes of glass and plastic often break upon freezing.

2. Urine

The urine samples may often be stored in the presence of a preservative (e.g. 1 mL 6 mol HCl or HNO_3 per litre urine, depending on the analytical methodology).
Note: no acidification of urine samples for analysis of proteins e.g. beta 2 microglobulin.

Specify density, creatinine concentration, urinary infections, nitrite test, stick tests,urinary proteins, volume, time of day, daily work schedule

2.1. Equipment and cleaning procedures

Cleaned polythene or other plastic container, specify brand of container and material it is made of, content of container.

Cleaning: a very thorough cleaning consists of a sequence with 30 % H_2O_2, detergent, water, high purity (1+10) 65% HNO_3 and (1+10) 96% H_2SO_4, and double-distilled water, steam cleaned;
depending on the element a simple acid rinse (10% HNO_3) followed by 3 times distilled water may be sufficient;
the blank value has to be checked in a similar way as for blood samples (see above)

2.2. Urine collection

In case of a 24 h collection, dust falling into the container remains a potential hazard, as the cover must be removed several times during one day. Even voiding of urine from the body into the vessel again introduces some major risk of contamination, e.g. from clothes and skin, particularly in connection with occupational exposure. Some researchers advise to collect each void in separate containers.

The receptive is wrapped in a clean polyethene bag in between sampling sessions.

The subject must wash his/her hands before voiding.

2.3. Storage

It is advisable to sub-sample the urine (divide the urine specimen into aliquots) after vigorous shaking for 2 min, as soon as possible (e.g. within 24 h after the end of the collection period).
Usually, upon storage of urine, precipitation of salts and organic compounds occurs resulting in co-precipitation of several trace elements, including increased uncertainty of the measurements. The samples should be kept at 5°C in the refrigerator, and this only for a few days, e.g. during the sample collection period.

Specify storage temperature, duration of storage before subsampling

Part C: Post sampling steps for blood, its derivatives and for urine

1. Information on sample treatment and analytical methodology

Name of analyst(s)

Preparation of the samples: sample dilution, drying or lyophilization, sample digestion or complete mineralization, filtration, deproteinization, preconcentration, solvent extraction,

evaporation, hydride generation, any other step of the procedure, report on the experience you gained on the element.

Try to develop a good discipline for working "clean" with low and well defined blanks from the recipients and the reagents.

blank value:absolute amount measured, expressed in µmol/L blood, serum, urine, µmol/kg packed cells
calibrants used should be recorded
All the steps relating to the analysis of the element should be recorded as precisely as possible.

2. Measurement
analytical technique, procedure, equipment, calibration..

3. Quality assurance
It should be described how standard curves and controls are used as well as if samples are run singly or repeatedly.
Details are requested about the quality assurance programme and the date of the last one.

The analysis of a reference material certified for the trace element at almost the same concentration as present in the samples is desirable to harmonize the results and provide quality assurance. Certified reference materials (CRM) are, however, not available for all trace elements in blood, serum, packed cells and urine. When a CRM is lacking, then the laboratory should produce a control material and establish the trace element concentrations by having it analyzed by other laboratories.
Quality assurance of sampling and handling implies also the control of the analytical blank.

Describe the internal quality assurance procedure

Describe the external quality assurance procedure

4. Evaluation of data
Data handling should be addressed appropriately [117]. This includes calculations, corrections, adjustment to standard conditions, normalization of data, computer programs, checks of internal consistency, corrections for systematic errors.
The uncertainty and accuracy of the measurements should be stated.

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