

Recommendations of the International Union of Pure and Applied Chemistry concerning analytical quality criteria in the biological monitoring of toxic metals

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CORNELIS R. Recommendations of the International Union of Pure and Applied Chemistry concerning analytical quality criteria in the biological monitoring of toxic metals. *Scand J Work Environ Health* 1993;19 suppl 1:14–18. A working party of the Commission of Toxicology of the International Union of Pure and Applied Chemistry has been set up to establish criteria to ensure analytical quality in the biological monitoring of toxic metals. Although the project has not yet been completed, some useful guidelines have been produced. They pertain to the description of the subject (so as to allow classification into a given "reference group"), the collection, storage and sampling of body fluids for trace-element determinations, and criteria to ensure the application of the correct analytical procedure, together with a quality-assurance protocol. It was agreed that a dedicated sampling protocol is required for each trace element in a particular matrix. Although it is impossible to specify this protocol together with all subsequent procedures in precise detail, the members of the working party have laid down general rules to be followed. This paper summarizes what has been achieved to date.

Key terms: arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, selenium, zinc.

One of the present tasks of the Commission of Toxicology of the International Union of Pure and Applied Chemistry (IUPAC) is to make recommendations concerning analytical quality criteria in the biological monitoring of toxic metals. For this purpose a working party was set up consisting of A Cavalleri (University of Pavia, Italy), R Cornelis (University of Gent, Belgium), J Duffus (Heriot-Watt University, Edinburgh, United Kingdom), P Grandjean (University of Odense, Denmark), R Herber (University of Amsterdam, The Netherlands), B Heinzow (Institute for Environmental Toxicology of Schleswig-Holstein, Kiel, Germany), M Jakubowski (Institute of Occupational Medicine, Cracow, Poland), A Lambert (Institute for Reference Materials and Measurements, European Community Research Centre, Geel, Belgium), J Molin Christensen (Institute of Occupational Health, Copenhagen, Denmark), O Poulsen (Institute of Occupational Health, Copenhagen, Denmark), E Sabbioni (EC Research Centre, Ispra, Varese, Italy), D Templeton (University of Toronto, Canada), Y Thomassen (Institute of Occupational Health, Oslo, Norway), M Vahter (Institute of Environmental Medicine, Stockholm, Sweden), C Veillon (United States Department of Agriculture, Beltsville, Maryland, United States), and O Vesterberg

(National Institute of Occupational Health, Solna, Sweden). It is the intention of the Commission to provide an organizational system for element-specific sample collection and the handling of human blood (whole blood, serum or plasma, packed cells, or erythrocytes) and urine in a standardized format calling for the proper definition of the subject and the sample and suggesting harmonized procedures for collection, preparation, analysis, and quality control. The aim is to assist scientists worldwide in producing comparable data useful at the regional, national, and international level.

SCOPE OF THE PROJECT

The task of the working party is to draw up guidelines on how to define the subject and to suggest element-dedicated harmonization guidelines for assessing arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, selenium, and zinc with respect to sample collection, preparation, analytical methodology, quality assurance, and evaluation of the data.

Description of subject

A detailed description of the subject is the first prerequisite to allow classification into a particular group or "universe" on the basis of well-defined criteria for purposes of comparison. With the benefit of hindsight, the working party hopes to take into account all of the important factors that make it possible to decide whether the results of future investi-

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gations can be regarded as applicable to the same population or reference group.

The importance of completeness can be illustrated by a study of the concentration of vanadium in human serum, investigated in a group of 37 men and 37 women (1). The data are expressed in molar units, in accordance with recent IUPAC rules. Serum levels of vanadium varied for the 37 men between 0.47 and $18.43 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ (24 and $939 \text{ pg} \cdot \text{ml}^{-1}$). The distribution was not normal, but nevertheless 28 of the 37 values were below $1.77 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ ($90 \text{ pg} \cdot \text{ml}^{-1}$). The two men with the highest levels of vanadium were sampled twice. Whereas the values of the concentration of vanadium in the serum of one of the men were similar in both instances, namely, 5.10 and $5.59 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ (260 and $285 \text{ pg} \cdot \text{ml}^{-1}$), those from the other showed a four-fold increase, from 4.91 to $18.43 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ (250 to $939 \text{ pg} \cdot \text{ml}^{-1}$). Both men were suspected of being contaminated with vanadium. It was discovered that the second man was responsible for technical services in a caravan construction unit, involving the handling of tools made of chromium-vanadium alloy. He also admitted that he tightened these tools between his lips. The first blood sample, which gave a value of $4.91 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ ($250 \text{ pg} \cdot \text{ml}^{-1}$), was taken on his tenth day of hospitalization for eye surgery; the second, which gave a value of $18.43 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ ($939 \text{ pg} \cdot \text{ml}^{-1}$), was taken in the middle of a work-week. The first subject appeared to be a truck driver who did not change his work pattern between the two sampling sessions. It is assumed that the high vanadium content of diesel fumes was the cause of the high values in his case. The data for the women were closer to a normal distribution, with the exception of those for one woman; 36 of the 37 values fell between 0.314 and $1.04 \pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ (16 and $53 \text{ pg} \cdot \text{ml}^{-1}$), approximating to a normal distribution with a mean value of 0.61 ($\text{SD } 0.20$) $\pm 10^{-9} \text{ mol} \cdot \text{l}^{-1}$ [31 ($\text{SD } 10$) $\text{pg} \cdot \text{ml}^{-1}$]. Once the survey was completed, the investigators concluded that they were analyzing the serum from an ill-defined population.

In order to obtain better results in the future, the working party will focus on describing the most important parameters for biological variability. It is the opinion of the Commission of Toxicology that research on trace-element levels in biological fluids and tissues may yield many different "reference" values, which will be applicable to a particular group only.

The list of parameters will start with information about the identity of the subjects, the place where sampling is carried out, the time of collection, and the names of the medical personnel collecting the samples. Each sample will carry a code in accordance with EN 45000 recommendations.

The personal history of the subject will be described in detail so as to ensure that subsequent categorization into a well-defined group of persons will be possible. Information will be requested about eth-

nic origin, gender, date of birth, recent and past employment, workplace environment, smoking habits, and hobbies, not only of the subject but also of the family, so as to ensure that hidden exposures are not overlooked. Detailed descriptions of eating and drinking habits are considered highly important and will include an estimate of the amounts of the different foods and drinks consumed. Moreover, in the case of arsenic and mercury, not only the amounts, but also the different varieties of fish consumed and information on geographic origin of the fish are also important.

Last but not least, the area of residence of the subject can affect some trace-element concentrations in the human body. Residence in urban areas increases the content of lead in blood, living in the vicinity to cadmium-processing plants causes elevated cadmium levels, and the like. The questionnaire should also include a detailed medical history based on medical records and not on a personal account.

Sample collection

Clinical laboratories are well aware that serious errors can be introduced at all stages between the collection of the sample and the ultimate determination of the analyte.

The aim of what follows is to ensure that a correct approach is adopted when a trace-element analysis of biological fluids is embarked on. The list of recommendations is not exhaustive, but on the contrary is fairly simple, so that they can be followed in normal laboratory practice. No claim is made that applying these simple rules will lead to perfection.

Blood, serum or plasma, packed cells or erythrocytes

Essential equipment and cleaning procedures: collection tube. A collection tube free of trace-element contaminants, as claimed by some manufacturers, simply does not exist. The raw material, as well as the impurities present, will always release some atoms into the sample. Certain requirements can be suggested — for example, that the amount of the trace element contributed to the blood or its derivatives by the cleaned tube, including the stopper, should be low and accurately measured so that it can be reported as the blank value. Some recommendations that certain materials should not be used for specific elements can obviously be made. For example, no glass should be used for aluminum determinations in the serum of healthy individuals, no plastics with a cadmium softener for cadmium analyses, no zinc-doped stoppers for zinc determinations, and the like.

Ultimately, the analysts are responsible for testing their own tubes. A reliable test procedure would

consist of collecting and analyzing blood or plasma containing a certified concentration of the element of interest in a sample which has been stored for about a day in one of the tubes. If a correct result is obtained, the contamination can be considered negligible. This assumption presupposes that the analytical procedure is satisfactory, which again can only be proved by the analysis of blood certified to contain "reference" concentrations of the trace element in question.

An alternative test method would involve mild acid rinsing, followed by an analysis of the leaching solution. This procedure is completely different from the analysis of a body fluid. On the one hand, the acid concentration is too high, while, on the other, the many ligands present on proteins, such as albumin, which so avidly bind any trace elements that may happen to be present on the wall or in the material, are lacking. Depending on whether a certified reference material is available or not, it will be the user's decision to test the tubes by one or other of these methods.

As an illustration of the impact of possible contamination from the tube wall, the manganese contamination measured in serum collected in unwashed vials amounted to >100%, while after a single rinse more than 10% may still be added, whereas thoroughly cleaned vials yielded the correct value of $0.01 \pm 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ ($0.55 \text{ ng} \cdot \text{ml}^{-1}$). It should be pointed out that manganese is an extremely difficult element to handle as far as avoidance of contamination is concerned.

Versieck & Cornelis (2) have described a very reliable cleaning procedure for collection vials and other laboratory ware in class 100 circumstances. The cleaning procedure encompasses, among other things, leaching of the vials with analytical grade acids at an elevated temperature, rinsing with distilled water, followed by treatment with highly purified (1+10) nitric acid and (1+10) sulfuric acid, and double-distilled water, and ending with steam cleaning. This procedure is undoubtedly both too elaborate and too expensive for routine laboratory work and may be excessive for some trace elements.

Essential equipment and cleaning procedures: the needle. The use of stainless steel needles for the collection of blood for, for example, chromium, cobalt, nickel, and manganese determinations can be practically ruled out. Their replacement by siliconized needles for trace-element determinations may be highly misleading, since it is known that some types of needles are only siliconized on the outside to facilitate their introduction into the artery and the risk of contamination of the blood sample remains.

The elements most liable to contamination by an uncovered stainless steel needle are chromium, molybdenum, nickel, vanadium, and manganese. The

solution lies in the use of a polypropylene intravenous cannula, mounted on a trocar.

Essential equipment and cleaning procedures: anticoagulants. The use of anticoagulants is open to many objections. These objections can be illustrated by the analytical data for, for example, manganese in serum and plasma, for which the results are totally unacceptable, although for zinc the problem is smaller. As a general rule, the contamination hazard should be checked for each element under investigation, and the blank value due to the anticoagulant should be reported for each individual batch.

Blood collection and separation of serum and packed cells. The following procedure has been taken from Versieck & Cornelis (2):

1. The antecubital fossa of the arm is cleaned (ie, without the use of cottonwool) with double-distilled water and analytical grade ethanol and allowed to dry by evaporation.
2. A tourniquet is applied.
3. Both the physician and the nurse use talc-free latex gloves.
4. Venipuncture is done with a needle. If an intranule is used, the stylus of the catheter is withdrawn but the polypropylene catheter is left in place. With healthy adults, the first 25 ml of blood are preserved for clinical laboratory tests and are not used for trace-element analyses. The samples are collected in cleaned collection vials. Either blood is allowed to clot spontaneously (20 min at room temperature for blood in glass vials and 1 h in plastic), or in the presence of the anticoagulant. Samples are transported to the clean laboratory.
5. Serum is separated by centrifugation at 900 g for 20 min at room temperature. The serum is decanted and centrifuged for a second time at 900 g for 15 min to remove the remaining blood cells, decanted again and stored in clean, stoppered vials. Visibly hemolized samples cannot be used for analyses of trace-element content.

The risk of contamination of the blood sample through sweat from the skin of the donor or the hands of the physician should not be underestimated. Molin Christensen & Pedersen (3) reported that the nickel concentration of sweat is $0.074 \pm 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ ($4.4 \text{ ng} \cdot \text{ml}^{-1}$) versus $0.0043 \pm 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ ($0.25 \text{ ng} \cdot \text{ml}^{-1}$) in serum, and the chromium concentration of sweat amounts to $0.038 \pm 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ ($2.0 \text{ ng} \cdot \text{ml}^{-1}$) versus $0.0056 \pm 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ ($0.29 \text{ ng} \cdot \text{ml}^{-1}$) in serum.

Storage. The sample should be stored either in the refrigerator at $<5^{\circ}\text{C}$ or deep frozen at -20°C . The duration of storage may be element dependent, although the analytical uncertainty for most elements is expected to be too high for any difference to be discerned.

Urine

Although 24-h urine collection may be scientifically more interesting, spot samples are easier to obtain. The creatinine content of the urine samples must be monitored and their density measured. They should be stored in the presence of a preservative (eg, hydrochloric acid).

Equipment and cleaning procedures. A suitable collection vessel is a high-density polyethylene container, thoroughly cleaned according to the procedure already described for blood and its derivatives.

Urine collection. Although it may seem trivial, it is not necessarily true that the urine collected will provide a sample adequate for trace-element determinations. In the case of 24-h collection, dust falling into the container remains a hazard, as the cover must be removed several times during the day, usually not in dust-free rooms. The voiding of urine from the body into the vessel again introduces a major risk of contamination (eg, from clothes and skin).

Between sampling sessions, the receptacle should be wrapped in a clean polyethylene bag.

Storage. It is advisable to subsample the urine as soon as possible (eg, within 24 h after the end of the collection period), after vigorous shaking for 2 min.

A stability study of 15 elements in urine stored for 3 d at room temperature revealed no significant differences for cobalt, copper, manganese, selenium and zinc, nor for bromine, calcium, chlorine, chromium, cesium, iodine, potassium, sodium and rubidium (4). In contrast there appeared to be about a 15% loss of arsenic in the deposit or on the walls.

Postsampling steps for blood and its derivatives, and for urine

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, and hydride generation. All of these steps should be recorded as precisely as possible, and the blank values determined.

Measurement and calibration. A discussion of the advantages and disadvantages of the different detection methods is outside the scope of this paper. What is required is the correct use of the analytical methodology, which should have adequate specificity and sensitivity.

Quality assurance

Nowadays clinical laboratories are aware that their analytical procedures should be subject to the basic rules of quality control and quality assurance (5–8). The analysis of reference materials for blood, serum, packed cells, and urine, certified to contain trace elements at the same concentrations as those present in the samples, is essential in order to harmonize the results and provide quality assurance. Quality assurance of sampling and handling also implies the control of the analytical blank.

In his chapter on quality assurance, Friberg (7) makes several interesting remarks and recommendations on quality assurance and quality control for the biological monitoring of metals. There is no magic formula for assuring the quality of data. A critical approach, combined with quality-control exercises that are an integral part of the monitoring project itself, is a major asset in this endeavor.

Evaluation of data

Accuracy cannot be defined in absolute terms. The evaluation of the data will depend on their end use. A measurement process capable of producing the same value with an error of 10%, or even 20%, would be considered to be precise for ultra trace determinations (arsenic, cobalt, manganese, nickel, etc), but not for zinc, copper, and selenium.

TYPICAL RANGES OF CONCENTRATIONS

Very stringent criteria for the evaluation of literature data have recently been advocated by Vesterberg et al (9). Their work is well known as the TRACY project, a computer software package for rating published data according to an elaborate checklist. To my knowledge not a single publication has been graded at the highest level. It is therefore with a some trepidation that I present typical ranges of concentrations for trace elements in serum or plasma, packed cells or erythrocytes, whole blood, and urine in table 1. Values can be found for plasma or serum in a book by Versieck & Cornelis (2), for packed cells and urine in a review article by Versieck (10), for cadmium in blood and urine in the publications by Ducoffre et al (11) and Sartor et al (12), respectively, for mercury in subjects with unknown fish consumption in a paper by Brune et al (13), and for lead in whole blood in a paper by Ducoffre et al (14). If a hematocrit value of 45% is assumed, the mean trace-element level in whole blood can be estimated.

Table 1. Typical ranges of concentrations for trace elements in human serum or plasma, packed cells or erythrocytes, whole blood, and urine (2, 10, 11, 12, 13).

Element	Serum or plasma	Packed cells	Whole blood	Urine (24 h) ^a
Arsenic ^b	13 nmol · l ⁻¹	24 pmol · g ⁻¹	—	300 nmol · d ⁻¹
Cadmium ^c	2 nmol · l ⁻¹	—	10 nmol · l ⁻¹	8 nmol · d ⁻¹
Cobalt	3 nmol · l ⁻¹	3 pmol · g ⁻¹	—	20 nmol · d ⁻¹
Chromium	4 nmol · l ⁻¹	4 pmol · g ⁻¹	—	10 nmol · d ⁻¹
Copper	17 μmol · l ⁻¹	10 nmol · g ⁻¹	—	300 nmol · d ⁻¹
Mercury ^b	10 nmol · l ⁻¹	40 pmol · g ⁻¹	25 nmol · l ⁻¹	10 nmol · d ⁻¹
Manganese	9 nmol · l ⁻¹	300 pmol · g ⁻¹	—	10 nmol · d ⁻¹
Nickel	1 nmol · l ⁻¹	—	—	5 mmol · d ⁻¹
Lead ^d	20 nmol · l ⁻¹	—	380 nmol · l ⁻¹	—
Selenium ^b	1 μmol · l ⁻¹	3 nmol · g ⁻¹	—	400 nmol · d ⁻¹
Zinc	15 μmol · l ⁻¹	180 nmol · g ⁻¹	—	10 μmol · d ⁻¹

^a Large spread around values cited.^b Highly influenced by dietary intake.^c Highly dependent on smoking habits.^d Highly dependent on environmental exposure.

CONCLUDING REMARKS

The advice given by IUPAC is to define the subject as closely as possible and to scrutinize closely every step of the analytical procedure: presampling, sampling, sample manipulation, analytical method, measurement, calibration, evaluation, and quality assurance. The data presented should be as complete as possible; and the range covered, the 95th percentile, the test for normality, and the mean and standard deviation should be cited. The working party of the Commission of Toxicology will draw up guidelines for producing reliable clinical results for trace elements in blood and urine and will publish their conclusions, including a mastersheet, in the IUPAC journal *Pure and Applied Chemistry*. The aim is the harmonization of results worldwide, and the work is being carried out jointly by the International Commission on Occupational Health (15) and the European Community (16).

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