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CLINICAL CHEMISTRY DIVISION  
COMMISSION ON TOXICOLOGY\*

Working Party on Nickel Reference Levels†

### MEASUREMENT OF TOTAL NICKEL IN BODY FLUIDS

#### ELECTROTHERMAL ATOMIC ABSORPTION METHODS AND SOURCES OF PREANALYTICAL VARIATION

(Technical Report)

*Prepared for publication by*

DOUGLAS M. TEMPLETON

Department of Clinical Biochemistry, University of Toronto,  
100 College St., Toronto, Canada M5G 1L5

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# Measurement of total nickel in body fluids: Electrothermal atomic absorption methods and sources of preanalytic variation (Technical Report)

## *Synopsis*

Before attempting to establish reference concentrations of Ni in blood serum or plasma, a laboratory must be working comfortably at the 2 nmol/L level. To achieve this, scrupulous acid-leaching of all materials that come into contact with the sample is essential, and blood collection with non-metallic systems is advisable. A particle-free environment (clean-air laboratory) seems necessary. Values of Ni in serum above 1.7 to 5.1 nmol/L are suspect; values in the urine of people without occupational or other unusual environmental exposures are an order of magnitude higher. For reported concentrations to be meaningful, the reference population must be thoroughly described in terms of age, sex, place of residence, occupation, diet, alcohol and tobacco use, medications and medical history, as all such factors may influence the actual concentrations of Ni. Sampling procedures must be standardized (for instance for time of day, season, posture of the person, etc.) and reported. Storage, if necessary, should minimize opportunities for sample adulteration, and these must be evaluated empirically. Preparation of samples should also minimize processing; acidification or protein precipitation with ultrapure nitric acid are adequate for clear body fluids. Analysis by ET-AAS with Zeeman correction is the most useful approach currently available.

## 1. INTRODUCTION

Exposure to Ni is of occupational significance worldwide, occurring not only in those involved in Ni mining, smelting and refining, but also in such diverse settings as the petroleum and electronics industries. Nickel is ubiquitous in our environment; indeed, exposure of the general population through jewelry, coinage and dental alloys is well documented as a frequent cause of contact dermatitis [1, 2], and oral exposure can be greatly increased by leaching of Ni from plumbing fixtures and kitchen utensils. The International Agency for Research on Cancer (IARC) has recently concluded that "There is sufficient evidence in humans for the carcinogenicity of nickel sulfate. ... Nickel compounds are carcinogenic to humans (Group 1) [whereas] Metallic nickel is possibly carcinogenic to humans (Group 2B)" [3]. Nevertheless, exposure from natural sources is unavoidable.

There is also some evidence that Ni may be essential for human beings, as it is for numerous species of plants and lower animals [4]. Therefore it is of the utmost importance to measure concentrations of Ni in human body fluids, both in order to understand the natural biology of this element and as a reference point for monitoring undue exposures. It is a goal of the IUPAC Commission on Toxicology to recommend reference substance concentrations for Ni in human blood serum or plasma, whole blood, and urine. At present, the accuracy of all published values remains in some doubt, and all but a few must clearly be rejected. The aims

of this report are:

- i) to review the recent literature in order to determine whether an approximate range of Ni concentrations in body fluids can be recommended at this time;
- ii) to suggest a level of accuracy that any laboratory must achieve before it can seriously contribute to the effort of establishing reference ranges for Ni in serum or plasma;
- iii) to recommend conditions of sampling and storage that are necessary before any analytical method with adequate sensitivity can be used to establish meaningful values in a specified reference population; and
- iv) to describe current methods of measuring concentrations of Ni by electrothermal atomic absorption spectrometry (ET-AAS).

A companion document from this Commission will consider sampling criteria for trace elements in general. This report will deal with sampling conditions specific for Ni. Because statistical considerations for sampling are not specific for Ni they are not discussed here. Some general aspects of sample storage are discussed, again with reference to Ni. Concluding that ET-AAS is the best candidate reference method for Ni, we also review current methods of sample preparation for analysis of biological fluids for Ni by this technique.

Use of substance concentrations for reporting Ni should be encouraged. Nevertheless, with rare exceptions, analysts continue to report mass concentrations of Ni in body fluids in  $\mu\text{g/L}$ . Here we will retain this less preferable unit when quoting authors' original data, and include the conversion to molar units in parentheses based on an atomic mass of Ni of 58.69. A solution with a mass concentration of Ni of  $1.0 \mu\text{g/L}$  has a substance concentration of  $17.0 \text{ nmol/L}$ .

## 2. NI CONCENTRATIONS IN BODY FLUIDS

Over the past decade, in common with a number of other trace elements, the trend has been a progressive lowering of reported concentrations of Ni. Thus, the chronology reveals new record lows for the serum/plasma value in  $\mu\text{g/L}$  ( $\text{nmol/L}$ ) being set as follows (mean  $\pm$  s.d. [year] - see Table 1):  $1.3 \pm 0.37$  ( $22 \pm 6$ ) [1983],  $0.46 \pm 0.26$  ( $7.8 \pm 4.4$ ) [1984],  $0.14 \pm 0.09$  ( $2.4 \pm 1.5$ ) [1989]. We can conclude at the outset that the usual range of Ni concentration in serum is less than  $20 \text{ nmol/L}$  in healthy people with no history of excessive exposure, and that earlier higher values were a consequence of sample contamination, inadequate analytical sensitivity, or both. So, only the literature from 1983 onward need be seriously considered. We cannot now state a value of Ni in body fluids with much certainty. Examination of Table 1 suggests that the level of serum Ni is in the range of  $1.7$  to  $3.4 \text{ nmol/L}$ . Concentrations of Ni in urine have been measured consistently at more than  $17 \text{ nmol/L}$  and therefore are probably an order of magnitude higher than in serum. Intermediate values can be expected in blood. Before a laboratory can contribute to the goal of measuring concentrations of Ni in blood serum collected from a chosen reference population, it must work at the ultratrace level of  $2 \text{ nmol/L}$  and below. The accurate measurement of Ni in blood and urine should present less difficulty for analysts who successfully meet the challenge of serum analysis. This report considers approaches to sample handling that will help reduce the measured concentration in a zero reference (method blank) for Ni to one where its accurate measurement in serum is feasible. It also reviews factors that must be taken into consideration when a reference population is chosen for determining a reference value for Ni.

Table 1. Selected studies of nickel concentrations in body fluids

Ref.	Converted*† Value (nmol/L)	Subjects‡	Collection¶	Apparatus	Reagents	Manipulation	Clean Air	Notes
[5]	(S) 95 ± 20	10	NS	+	+	+	-	Examined materials & solutions for Contamination
[6]	(P) 22 ± 6.3 (B) 41 ± 8.5 (U) 19.4 ± 5.4	20 (Hx, alcohol, Tob., Rx, Occup.)	+(needle NS)	NS	NS	-	-	Ni-DMG extraction in MIBK
[7]	(B) 24.0 ± 6.3 (U) 39.1 ± 9.9 (Saliva) 42.7 ± 19.0	9 9 5	NS	+	+	-	- (?)	Ammonia buffer, DMG
[8]	(S) 7.8 ± 4.4 (B) 21.4 ± 5.6	39 (S), 30 (B) (Hx, Tob., Occup.)	+	+	+	+	- (?)	Deproteinized samples Thorough documentation of apparatus & solutions
[9]	(S) 4.8 ± 4.1 (B) 5.8 ± 4.8	30 (Hx, Rx, Tob., Occup.)	+	+	NS	+	-	Assessed confounding factors - time of serum separation, sex, exercise, post-prandial & diurnal fluctuation
[10]	(S) (median 17) (<10 - 51)	71 (University Employees)	+(SS needle)	+	+	+	-	Undigested serum mixed with Triton X-100; Assessed contamination in sampling devices, containers, SS needles
[11]	(S) 128 ± 53	18	+(butterfly)	NS	NS	+	-	Deproteinized
[12]	(S) 4.25 ± 1.7 (U) 14 ± 8.5	10 10	+	+	+	- (Pronase) - (Sonication)	-	
[13]	(S) 11 ± 6.0	6	NS	+	+	+	-	Serum diluted with HNO <sub>3</sub> /Triton X-100 for direct analysis
[14]	(S) 95 ± 14	30 (fasting)	+	NS	NS	NS	-	
[16]	(S) 7 (3 - 9)	20	+(SS needle vs. teflon cannula)	+	+	+	-	Deproteinized serum; compared needle vs. cannulae, open vs. closed collection
[17]	(S) 3 ± 3 (U) 25 ± 25 nmol /g creat.	43 (Hospital workers)	+	+(by ref.)	+(by ref.)	+(by ref.)	- (?)	Deproteinized serum
[18]	(S) 2.4 ± 1.5 (mean ± 2 s.d)	38 (Physical & lab work; fasting)	+(SS needle)	+	+	+	+	Clean room. Serum:HNO <sub>3</sub> /Triton X-100 3:1; multiple injection. Materials contacting sample acid leached; Needle blood rinsed. Contamination control discussed.



### 3. PREANALYTIC SOURCES OF VARIATION

It is not yet known whether Ni is essential for human health, or the limits within which its concentration in blood plasma may be regulated in chronic exposure. This leaves open the question of whether there is an expected concentration that is homeostatically regulated, and the extent to which concentration of Ni is determined by individual circumstances. However, ample evidence is presented below to demonstrate that increased Ni intake occurs for a variety of reasons, and can increase concentrations in blood and urine. Thus all factors potentially affecting Ni exposure should in principle be documented for the reference population. Furthermore, factors such as age, sex and general health may have poorly understood effects on Ni concentrations, and must be carefully reported.

#### Age and sex

Apart from possible differences due to pregnancy and parturition (see below), sex differences in serum Ni concentration have not been demonstrated. Hohnadel et al. [21] reported higher concentrations of Ni in sweat from females [ $131 \pm 65 \mu\text{g/L}$  ( $2.23 \pm 1.11 \mu\text{mol/L}$ );  $n=15$ ] than from males [ $52 \pm 36$  ( $0.89 \pm 0.61$ );  $n=33$ ] as measured by flame atomic absorption spectrometry. However, these values are clearly too high, and were compromised by the techniques available at the time; Ni concentrations in sweat of 10 healthy people (sex not specified) has subsequently been reported to be  $4.4 \pm 2.5 \mu\text{g/L}$  ( $75 \pm 37 \text{ nmol/L}$ ) [12]. Furthermore, there is no reliable information on age effects, particularly in the extreme paediatric and geriatric populations. No significant differences in Ni in serum or blood of healthy adults were found between groups over and under 40 years of age [9]. Until these factors are investigated, reference populations should be well characterized with respect to their age and sex distribution.

#### Geographic location

A number of factors in the human environment determine exposure to Ni, and these must be reported in any purported reference study. Ni in soil varies between about 0.09 and 9  $\mu\text{mol/g}$  depending on geological factors, while various unpolluted water supplies generally have Ni concentrations in the range 0.02 to 0.85  $\mu\text{mol/L}$  [22]. Water supplies in industrially polluted areas can have more than 20  $\mu\text{mol/L}$ . The latter can increase to the mg/L range in waters. In the U.S.A., rural air typically contains Ni at a concentration of 0.10  $\text{nmol/m}^3$ , while in urban air the concentration is about 0.43  $\text{nmol/m}^3$  [23, 24]. Values up to 2.9  $\text{nmol/m}^3$  occur in some industrial centers [24]. Hopfer et al. [17] have compared serum Ni concentrations in healthy hospital workers in Hartford, CT, U.S.A. ( $n=43$ ) and Sudbury, Ont., Canada ( $n=22$ ). The Sudbury cohort lives in a community of nickel mines and smelters, and is exposed to higher concentrations of Ni in tap water and soil. These people had higher concentrations of Ni in their serum [ $0.6 \pm 0.3 \mu\text{g/L}$  ( $10 \pm 5 \text{ nmol/L}$ ) in Sudbury versus  $0.2 \pm 0.2 \mu\text{g/L}$  ( $3.4 \pm 3.4 \text{ nmol/L}$ ) in Hartford,  $p < 0.05$ ].

#### Occupation

Because of the high worldwide production and use of Ni compounds, occupational exposure is widespread. Highest exposures occur in Ni refining and electroplating, arc welding and Ni-Cd storage battery production. Industrial exposure also occurs from the production of Ni alloys, its use as a catalyst for hydrogenation of organic compounds, as a fluid catalyst in the petroleum industry, as a pigment for ceramics and glasses, as a mordant for dyeing cloth, and in electronic components and magnetic tapes [23, 24]. A study of 8 workers exposed to Ni compounds in the workplace revealed a Ni concentration of 11.5  $\mu\text{mol/m}^3$  in air, whereas 103 welders breathed air with a mean Ni content of 1.6  $\mu\text{mol/m}^3$  [25]. In the

first group, high concentrations of Ni in urine correlated with high concentrations in blood; no such correlation was found in the welders. Occupational exposure can therefore have significant though unpredictable effects on Ni concentrations in biological fluids, and must be well documented.

#### Diet

A major source of Ni exposure is dietary, accounting typically for at least 1.7  $\mu\text{mol/day}$  of ingested Ni [2]. However, certain diets can result in considerably higher intakes. While meats have Ni contents in the range 1.0 to 7.2 nmol/g, the corresponding range in fruits and vegetables is 0.9 to 150 nmol/g [22]. Therefore, vegetarian diets will lead to higher Ni intake. Nuts (e.g. cashews 85 nmol/g), spinach (150 nmol/g dry weight), tea leaves (77 nmol/g fresh weight) [22], cocoa (167 nmol/g), soy products and oatmeal [26] are especially high in Ni. Diets rich in these foods can cause high rates of urinary excretion [26].

#### Tobacco and alcohol

Cigarettes each contain 34 to 105 nmol of Ni, and 10 to 20 % is released in inhaled smoke [23]. The daily inhaled dose of Ni has been estimated to be 68 nmol for a smoker of 20 cigarettes per day [2]. This is probably insignificant in relation to intake from food, drinking water and other sources. However, smoking may also increase the uptake of Ni and Cr from other sources in exposed workers, giving rise to elevated urinary levels far in excess of those accountable for by metals inhaled from the cigarettes themselves [27].

Because of the few reliable measurements of concentration in serum to date, there is insufficient information to allow comment on the effects of alcohol consumption on Ni status. The numerous effects of chronic alcohol consumption on general physiology, health, nutrition and body composition dictate careful documentation of patterns of alcohol use of any reference population. The Ni content of beer and wine is generally in the range 0.17 to 3.4  $\mu\text{mol/L}$  [22], although the diuretic effect of intake of large volumes of these beverages on Ni excretion is not known, and their impact on Ni concentrations is therefore hard to predict.

#### Health status

A number of systemic diseases may alter Ni concentrations in body fluids. Patients in renal failure have impaired excretion of Ni. Chronic haemodialysis compounds the problem, and hypernickelaemia in such patients is well documented. Several of the more reliable reports of Ni concentrations in serum of healthy adults have included comparative data on haemodialysis patients [11, 17, 18, 28]. Values in the renal patients were  $3.71 \pm 1.54$  ( $63.2 \pm 26.2$ ) versus  $0.44 \pm 0.18$  ( $7.5 \pm 3.1$ )  $\mu\text{g/L}$  (nmol/L) [11],  $5.4 \pm 2.1$  ( $92 \pm 36$ ) versus  $0.3 \pm 0.2$  ( $5.1 \pm 3.4$ )  $\mu\text{g/L}$  (nmol/L) [28],  $7.2 \pm 2.2$  ( $123 \pm 37$ ) versus  $0.2 \pm 0.2$  ( $3.4 \pm 3.4$ )  $\mu\text{g/L}$  (nmol/L) [17], and  $6.38 \pm 3.36$  ( $108 \pm 57.1$ ) versus  $0.14 \pm 0.09$  ( $2.38 \pm 1.53$ )  $\mu\text{g/L}$  (nmol/L) [18]. Thus increases of nearly 50-fold may occur in a population undergoing dialysis. Acute myocardial infarction is associated with an increase in serum Ni in the following 72 h [peak,  $3.0 \pm 3.4$  ( $51 \pm 58$ )  $\mu\text{g/L}$  (nmol/L) versus  $0.28 \pm 0.24$  ( $4.76 \pm 4.08$ )  $\mu\text{g/L}$  (nmol/L) in healthy adults] [9]. The same study also noted higher values in unstable angina pectoris [peak concentration  $1.4 \pm 0.9$   $\mu\text{g/L}$  ( $24 \pm 15$  nmol/L)]. Christensen & Pedersen [12] found higher values in serum Ni in 10 rheumatoid arthritis patients [ $0.64 \pm 0.3$   $\mu\text{g/L}$  ( $11 \pm 5.1$  nmol/L)] compared to 10 healthy controls [ $0.25 \pm 0.1$   $\mu\text{g/L}$  ( $4.25 \pm 1.70$  nmol/L);  $p < 0.025$ ]. The same study also reported increased Ni in urine [ $2.26 \pm 1.8$   $\mu\text{g/L}$  ( $38.4 \pm 30.6$  nmol/L) versus  $0.82 \pm 0.5$   $\mu\text{g/L}$  ( $13.9 \pm 8.5$  nmol/L)] and sweat [ $10.6 \pm 6.7$   $\mu\text{g/L}$  ( $181 \pm 114$  nmol/L) versus  $4.4 \pm 2.5$   $\mu\text{g/L}$  ( $75 \pm 43$  nmol/L)], both significant at the level of  $p < 0.025$ .

Other putative medical causes of hypernickemia are poorly documented and are found in publications reporting highly suspect 'normal' values; for a critical discussion see Versieck & Cornelis [29]. Parturition may lead to modest increases in Ni in maternal serum, perhaps due to release of Ni from the placenta. Earlier surprising reports of dramatic increases [30], however, have been refuted by the more reliable data of Nomoto et al. [31], who report concentrations of  $2.1 \pm 0.90 \mu\text{g/L}$  ( $36 \pm 15 \text{ nmol/L}$ ) in healthy adult women versus  $2.85 \pm 0.67 \mu\text{g/L}$  ( $49 \pm 11 \text{ nmol/L}$ ) 60 min post partum. There is little reliable data to support proposed changes in Ni status in diabetes, hepatobiliary disease, cancer or infectious processes. Urinary tract infections, often overlooked, should always be diagnosed at the time of collection and, if present the sample should be discarded.

#### **Medication and iatrogenic exposure**

Certain medications can affect Ni concentrations in body fluids. Disulfiram increases Ni uptake by chelating it in the gut [24]. Oral contraceptives were considered a potential source of differences in serum Ni concentrations among women [21]. Although the study reported no significant differences, it was conducted without the benefit of present technology, and concentrations above 35 nmol/L were found in both groups. Contamination of intravenous fluids presents a risk of triggering allergic reactions to Ni; concentrations of up to  $1.7 \mu\text{mol/L}$  Ni have been found in various lots of commercial human serum albumin [32]. Such iatrogenic exposure might account for the high concentrations in hospitalized patients undergoing invasive treatment, for example the increases that were noted in cardiac patients. Leaching of Ni from orthopaedic prostheses is a potentially insidious route of exposure [1, 33, 34]. Dental prostheses must also be considered. A 30-fold rise in concentration of Ni in saliva, attributable to leaching from dental posts of white gold, was found in a patient with extensive oral surgery [35]. Corrosion of pacemaker wires is another potential source of Ni [24].

#### **Seasonal and diurnal variation**

Variation in exposure may be seasonal. In one report, the Ni content of urban air rose from  $0.29 \text{ nmol/m}^3$  in summer to  $0.43 \text{ nmol/m}^3$  in the winter [24], probably through an increase in the burning of fossil fuels in the winter months. Leach et al. [9] found no diurnal variation in serum Ni in two volunteers. Although concentrations in urine fluctuated, they did not appear to do so systematically. In the absence of further data on circadian variation, standardized time of collection (e.g. early morning, fasting) is recommended.

### **4. SAMPLE COLLECTION FOR Ni MEASUREMENT**

#### **Preparation of skin**

Careful washing of the skin to remove air-borne dust is mandatory, in order to avoid contamination. A final wash with ethanol and drying by evaporation has been recommended [8]. The washing procedure also serves to remove sweat from the skin. The Ni concentration in sweat is about 5 times [12] or even 10 to 20 times that of urine [24]. Contaminant Ni, and also Cu, Zn and Pb, were found in sweat collected in a sauna [21]. Evaporation increases the concentration of Ni on the skin surface [36]. Similarly, the person taking the sample should wear plastic gloves to avoid contamination from sweat on the hands, and the gloves should not be of the talc-powdered variety.



### Selection of needle

Recognizing the potential for leaching Ni from stainless steel, the IUPAC reference method for Ni in serum recommends the use of Teflon or polyethylene cannulae for blood collection [37]. This practice seems well founded, and most studies in recent years have adopted this practice (see Table 1). Versieck et al. [38] drew 20 mL portions of blood at 37 °C through a steel needle (19G-11/2) after irradiation. Neutron activation analysis revealed substantial leaching of Ni into the sample. An addition of 1.28  $\mu\text{mol/L}$  to the first portion decreased in subsequent portions, but was still 0.22  $\mu\text{mol/L}$  by the fourth. Clearly these values suggest the need for extreme caution, although leaching facilitated by irradiation damage to the needle cannot be ruled out in this study. Sunderman Jr. et al. [8] collected blood from one arm of 21 people through a stainless steel needle (Monoject; mass fraction of Ni 0.091) and through an intravenous polyethylene cannula in the contralateral arm. The mean Ni concentration in serum from the stainless steel needle was  $0.74 \pm 0.25 \mu\text{g/L}$  ( $12.6 \pm 4.3 \text{ nmol/L}$ ), versus  $0.37 \pm 0.18 \mu\text{g/L}$  ( $6.3 \pm 3.1 \text{ nmol/L}$ ) with the cannula. The mean paired difference was  $0.38 \pm 0.23 \mu\text{g/L}$  ( $6.5 \pm 3.9 \text{ nmol/L}$ ). Blood from the cannula was collected into acid washed plastic syringes, whereas blood from the needle was collected into blue-stoppered "metal-free" Vacutainer tubes (Becton-Dickinson), so it was really the entire collection system that was being compared and difference cannot be attributed to the needle alone. Collection of cerebrospinal fluid with stainless steel needles was shown to result in excessive contamination precluding analysis for Ni [39]. Platinum needles with gold-plated Luer hubs have been fabricated for Ni measurement in serum samples [40], but are quite expensive and not generally available. Siliconized needles may in some cases be sufficiently hydrophobic to prevent leaching of Ni into blood [29]. However, extreme caution must be exercised in relying on siliconization. For example, Becton-Dickinson single-use hypodermic needles (e.g. No. 5175), often described by analysts as siliconized, are actually silicon-coated only on the outside, for lubrication and comfort of the patient.

Important exceptions to the prudent use of plastic cannulae are to be found. Bro et al. [16] compared concentrations of Ni in blood serum of 20 healthy adults after collection into acid-washed polyethylene syringes through either stainless steel syringes (Terumo; 18 G, siliconized) or 17 G or 18 G teflon cannulae. The first 5 mL of blood was discarded. Confidence intervals of 95 % for differences between the samples from the needle and either cannula were calculated, and no significant differences were found. A mean Ni concentration of  $0.4 \mu\text{g/L}$  ( $6.8 \text{ nmol/L}$ ) was reported [range 0.2 to  $0.5 \mu\text{g/L}$  ( $3.4$  to  $8.5 \text{ nmol/L}$ )]; a similar value was found with an open collection system (teflon cannula + acid-washed polyethylene tube). Nixon et al. [18] employed a class 100 clean lab and leached all plastic labware with nitric acid. Blood was drawn through a stainless steel needle (Becton-Dickinson No. 5175) after discarding the first 3 mL. The Ni concentration in serum obtained by these authors,  $0.14 \pm 0.09 \mu\text{g/L}$  ( $2.4 \pm 1.5 \text{ nmol/L}$ ), is among the lowest values yet reported. The use of a flushed stainless steel needle remains to be re-evaluated under the otherwise rigorous conditions employed by this group.

### Blood collection

General awareness of vascular physiology is needed to collect reliable samples. Changes in posture may affect intravascular volume. Pooling of blood in the lower extremities on standing can cause a 10% increase in non-diffusible components in the upper extremities [36], as well as an increase in apparent haematocrit. Because a major fraction of Ni is bound to the macroglobulin nickeloplasmin, as well as to albumin and other proteins [24], an

increase in these non-diffusible components results in an apparent increase in concentration of Ni. Furthermore, because the concentration of Ni in whole blood is several times that in serum [8], increases in haematocrit will be reflected in high concentrations in blood. Application of a tourniquet can force water from the vascular space, further increasing the apparent concentration of non-diffusible components [36]. Blood should be collected with the donor reclining and use of a tourniquet should be avoided. Use of a larger gauge needle or cannula (preferably 19 gauge or larger) will reduce the volume of the sample contacting the surface, and will minimize haemolysis from turbulent flow when blood components (e.g. serum, red cells) are to be separated. Tourniquets and rapid withdrawal of blood also contribute to haemolysis. The IUPAC Reference Method [37] calls for collection via polyethylene cannula (discarding at least the first 2 mL) into a polypropylene syringe and transfer to a polyethylene tube. An appealing alternative, dropwise collection directly into the tube under particle-free air flow, may not be practical with any but the most dedicated volunteers.

Blood should be allowed to clot under standardized conditions (e.g. 45 min at 20 °C) and serum separated at 900 x g for 15 min. Prolonged clotting time, excessive centrifugal forces and delays in separating serum from the cell pellet will all contribute to haemolysis. The use of plasma affords immediate and gentler separation, but addition of anticoagulants to the collection tube probably poses insurmountable difficulties for analysis at the ultratrace level. Heparin is a polyelectrolyte that avidly binds divalent cations, including Ni [41], and is difficult to purify. Other Ca<sup>2+</sup> scavengers (citrate, oxalate, EDTA, fluoride) pose a high risk of contamination [36].

#### **Washing of labware**

All surfaces coming into contact with the sample for ultratrace analysis must be scrupulously cleaned. This is of course true when the analyte is Ni in body fluids. A practical illustration of potential pitfalls is given by Nixon et al. [18]. Forty-seven serum samples were collected in acid-washed Monovette syringes and handled throughout for analysis by ET-AAS in a clean room. However, they were either transferred into non-acid-leached Sarstedt tubes, or aliquoted with non-acid-leached pipette tips. Against a reference concentration of Ni of  $0.14 \pm 0.09 \mu\text{g/L}$  ( $2.4 \pm 1.5 \text{ nmol/L}$ ) for the lab, failure to leach the tubes produced a value of  $0.93 \pm 0.64 \mu\text{g/L}$  ( $16 \pm 11 \text{ nmol/L}$ ) ( $n=22$ ), whereas unwashed pipette tips gave a value of  $3.3 \pm 4.0 \mu\text{g/L}$  ( $56 \pm 68 \text{ nmol/L}$ ) ( $n=25$ ). Moody and Lindstrom [42] have compared the amount of Ni leached from several types of plastic and Teflon, by hydrochloric and nitric acids (Table 2). Although nitric acid always leached more Ni, the relative efficiencies of the two acids differed with the material, and the authors recommend sequential use of both acids. They have also recommended that final rinsing and soaking should be with the purest water available, although reagent-grade acids are adequate for the leaching steps. They further recommend storage of vessels for several weeks (until use) filled with ultrapure water, to allow the cleaning process to continue.

Guidelines for cleaning labware for trace and ultratrace metal analysis can be found in many excellent sources. Some are compiled in Table 3. A few make specific comments about Ni, but one is best advised to follow general principles and err on the side of caution. Any of these precautions can be relaxed only when the impact of their omission on the analytical result has been thoroughly evaluated.

**Table 2** - Amount of Ni leached from plastics (nmol/m<sup>2</sup>) during 7 d of exposure to acid (volume fraction in water 0.5) at room temperature (80 °C for Teflon).

Acid	Teflon FEP	Polyethylene (linear)	Polyethylene	Polycarbonate
HCl	140	140	50	50
HNO <sub>3</sub>	340	270	85	120

**Table 3** - Some protocols for cleaning of sample vessels for ultratrace analysis (AR - Analytic reagent grade; DD - distilled, deionized).

Ref.	Intended sample	Vessel	Pre-washing	Acid washing	Duration	Rinsing	Drying
[42]	Aqueous	Plastics, Teflon		1:1(v/v) HCl, 1:1(v/v) HNO <sub>3</sub> ; AR; RT (80°C for Teflon)	7d each, rinse between	Ultrapure water, soak as long as possible	Particle-free environment
[43]	Blood, Urine, Serum	Glass & Plastics	Non-ionic detergent	6 mol/L HCl (AR) 1 mol/L HCl (DD)	7 d each, rinse between	Distilled, deionized water	Aluminum oven, vortex with 6 mol/L HCl & water before using
[37]	Serum	Polyethylene	Hot detergent, tap water rinse, then deionized water x 6	Conc. HCl fumes, then dilute x 40	1 h in fumes, 20 min after dilution	Deionized water x 5, then Ultrapure water x 2	Oven dry 110°C in polyethylene canister, lid ajar
[29]	Serum	High-purity quartz, Teflon stoppers	Distilled water, 30% (v/v) H <sub>2</sub> O <sub>2</sub> for 2 d, rinse with 2 x quartz-distilled water	1:1(v/v) H <sub>2</sub> SO <sub>4</sub> :HNO <sub>3</sub> (both concentrated, Ultrapure)	Boil x 8 h	Quartz DD water, rinse, boil 2 x 8 h, steam clean 6-8 h	Oven dry, 55°C on Teflon foil or polypropylene tray
[44]	Natural waters	Linear polyethylene		1.5 mol/L HNO <sub>3</sub> (AR)	7 d minimum	Rinse with sample at least x 4	
[18]	Serum	Plastics, Teflon		dilute HNO <sub>3</sub>	Until use	Triply distilled water	Particle-free air

## 5. STORAGE OF SAMPLES FOR ANALYSIS

When considering long-term storage of a sample, the analyst must be aware of the risk of loss or addition of the analyte by adsorption to, or leaching from, the storage container; water loss from poorly sealed or permeable containers; and changes in sample homogeneity, for instance formation of a precipitate in stored urine that may adsorb Ni. The chance of leaching imposes the same considerations as in sample collection, although criteria may be even more stringent because of prolonged contact with the container. Adsorptive losses of Ni to most plastics are probably not significant. Stoeppler [22] found no adsorption of radioactivity onto the walls of polyethylene bottles when  $^{63}\text{Ni}$  was added to freshly voided urine. No adsorptive losses were observed over 6 months when urine was spiked with small amounts of Ni and stored at 4 °C (container not specified) [36]. The IUPAC reference method for Ni in urine recommends storage of urine at -20 °C in screw cap polypropylene tubes, after acidification with nitric acid [37]. Stoeppler has also reported adsorption of  $^{63}\text{Ni}$  to the sediment that forms when urine is stored. This occurred even when the sample was acidified, accounting for about 6% of the Ni at pH 6 and about 1% at pH 1 [22]. Sunderman Jr. [45] recommends acidification by addition of 10 mL  $\text{HNO}_3/\text{L}$  urine and storage at 4 °C for up to a week (-20 °C for longer storage). Medium- to long-term storage of urine at -20 °C without acidification is preferred by some authors [46].

The rate of water loss from various Teflon and plastic containers was investigated by Moody and Lindstrom [42]. Annual losses varied from 0.03 to 0.05% from polypropylene and 0.05% from Teflon FEP, to 1.6 to 2.0% from polycarbonate. Losses from transpiration versus around the closure were not distinguished. If long-term storage is necessary, use of well-sealed polypropylene or Teflon vessels should be considered, and surface area for leaching and adsorption should be minimized by avoiding containers that are larger than necessary.

## 6. SAMPLE PREPARATION FOR ATOMIC ABSORPTION SPECTROMETRY FOR NI IN SERUM AND URINE

By far the most widespread method available to many investigators wanting to measure Ni is electrothermal atomic absorption spectrometry (ET-AAS); cf. Table 1. Numerous other methods have been reviewed [22, 47, 48]. Though definitive methods based on isotope-dilution mass spectrometry will undoubtedly be developed, suggested reference levels will continue to be based on ET-AAS. The requirements for sample processing for this technique in some ways dictate short-term handling and pre-storage conditions. For example, if urine is to be acidified for storage and later analysis, an acid compatible with the analysis would be chosen. As another example, if serum is to be wet ashed for analysis, gratuitous transfer to an intermediate storage container may be undesirable. Therefore, it is appropriate in a discussion of sampling and storage for Ni measurement to consider also sample preparation for ET-AAS.

The earlier IUPAC reference method for Ni in serum and urine [37] involved complexation of Ni in an acid digest with ammonium pyrrolidin-1-yl-dithioformate followed by extraction with 4-methylpentan-2-one. Advances in ET-AAS have rendered this extensive manipulation unnecessary. Sunderman Jr. [45] has recommended protein precipitation with nitric acid and heat for analysis of blood, serum, cerebrospinal fluid and saliva, and acidification with dilute nitric acid for urine. In general, commercially available ultrapure nitric acid (e.g. J.T. Baker, Merck "Suprapur") is adequate without further purification [37], and in general does not contribute to the

blank if used sparingly [22]. A list of earlier dry-ashing procedures, and of wet-ashing in  $\text{HNO}_3\text{-HClO}_4$  mixtures has been compiled by Stoeppler [22]. The IUPAC reference method for Ni required complete digestion of both serum and urine with an equal volume of  $\text{HNO}_3\text{:HClO}_4\text{:H}_2\text{SO}_4$  in volume ratios of 0.6, 0.2 and 0.2, respectively. Decomposition is accomplished in open borosilicate tubes with stepwise increases in temperature over 4.5 h, finishing at 300 °C with evaporation of all but  $\text{H}_2\text{SO}_4$ . Andersen et al. [13] diluted serum with  $10^{-3}$  mol/L  $\text{HNO}_3$  (volume ratio 0.5) in Triton X-100 (volume fraction 0.001) for direct analysis; Drazniowsky et al. [10] simply used a dilution with 20 mL/L Triton X-100 (volume ratio 0.5). Urine was analyzed after dilution with an equal volume of 3 mol/L  $\text{HNO}_3$  in aqueous Triton X-100 (volume fraction 0.005) [49]. Nixon et al. also used a diluent of  $10^{-3}$  mol/L  $\text{HNO}_3$  in aqueous Triton X-100 (volume fraction 0.001), and found no increase in blank values when three injections of 50  $\mu\text{L}$  were made into the furnace instead of one. Detergent serves as a wetting agent and reduces carbon build-up in the furnace, and in these studies Triton X-100 appears free of significant contamination. Nevertheless, it must always be considered as an additional source of potential contamination. The recommendations of Sunderman Jr. [45] seem the safest, although decreased recovery because of precipitation of protein-bound Ni must be addressed. This appears to be a theoretical consideration; acidification should be sufficient to displace Ni from binding sites on known proteins. Concentrated nitric acid (50  $\mu\text{L}$ ) is added with vortexing to 1 mL serum and the mixture is heated to 70 °C for 5 min in a stoppered tube. The clear supernatant is analysed after centrifuging. Urine is diluted with two parts of ca. 0.2 % (w/v)  $\text{HNO}_3$  and centrifuged only if turbid.

## 7. NI MEASUREMENT BY ATOMIC ABSORPTION SPECTROMETRY

This discussion focuses on ET-AAS as the most important and generally available approach for measurement of Ni in body fluids. The preceding sections have dealt with pre-analytical considerations applicable to this technique. Several technical improvements in the past decade have lowered detection limits below the presumed concentration of Ni in most or all body compartments. These include greater instrument sensitivity, the implementation of Zeeman background correction, and furnaces that provide rapid temperature rise during atomization [8, 47].

**Table 4** - Temperature programs for Ni measurement by Zeeman-corrected ET-AAS. Data in columns (i) to (iv) are from refs. [8, 13, 16, 18], respectively.

	T ( °C)				Ramp (s)				Hold (s)				Gas
	(i)	(ii)	(iii)	(iv)	(i)	(ii)	(iii)	(iv)	(i)	(ii)	(iii)	(iv)	
Dry*	100,	100,	100,	-	1,	40,	10,	-	1,	20,	80,	-	on
	140,	-,	140,	120	60,	-,	60,	1	10,	-,	10,	10	on
	190,	200,	190,	160	30,	60,	30,	70	5,	10,	5,	5	on
Char/ash	-,	-,	-,	700	-,	-,	-,	30	-,	-,	-,	10	on
	1200,	1250,	1100,	1500	80,	30,	80,	10	50,	20,	50,	25	on <sup>‡</sup>
Atomize	2600,	-,	2500,	-	0,	-,	0,	-	5,	-,	5,	-	off <sup>‡</sup>
	-,	2700,	2700,	2750	-,	0,	1	,0	-,	6,	3,	5	off
Clean	2700,	2800,	-,	2800	1,	1,	-,	1	3,	2,	-,	2	on

\*These steps are repeated with a second sample introduction in study (iv).

<sup>‡</sup>In study (i), the gas flow is not turned off but is reduced from 300 mL/min to 30 mL/min for the last 5 s at 1200 °C and for the atomization step.

**Table 5** - Performance characteristics of several protocols for analysis for Ni in blood serum by Zeeman-corrected ET-AAS.

Ref.	Serum:Diluent	Sample vol. ( $\mu\text{L}$ )	Detection Limit (nmol/L)	Within-run imprecision (c.v.)	Long term imprecision (c.v.)
[8]	1:0.05	50	0.85*	3.4% at 6.8 nmol/L	8.1% at 6.0 nmol/L
[13]	1:1	50	1.53‡	-	-
[16]	1:0.05	60	-	8% at 12 nmol/L	11% at 12 nmol/L
[18]	1:0.33	2 x 50	1.0‡	3.2% at 17 nmol/L	-

\*95% confidence level

‡3 standard deviations above the blank

Four studies [8, 13, 16, 18] were selected from Table 1 on the basis of i) the apparent ability of the authors to approach the putative target value, ii) the reporting of analytical performance and experimental detail, and iii) geographic diversity. These four studies from Europe [13, 16] and the U.S. [8, 18] all used Zeeman-corrected ET-AAS and Perkin-Elmer instruments (either the PE 5000 or PE 3030). Similar temperature programmes were used (Table 4); there is no reason to suggest that the slight differences were of analytical significance.

The various methods of sample preparation result in different dilutions of serum, with a consequent need to introduce different volumes into the graphite furnace. Table 5 shows that when serum was deproteinized by the addition of small volumes of concentrated acid, analysis of 50  $\mu\text{L}$  was feasible; with dilution, multiple injections may become necessary (cf.[18]). Within-run and long-term imprecision with coefficient of variation in the range 5% and 10%, respectively, was generally attainable, with detection limits lower than 1.7 nmol/L.

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