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# Reference Materials for the Determination of Trace Elements in Biological Fluids

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Analytical Chemistry Division
Institute for Materials Research
National Bureau of Standards
Washington, D.C. 20234

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Final Report

Prepared for
**Division of Laboratories and Criteria Development**
**National Institute for Occupational Safety and Health**
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U. S. DEPARTMENT OF COMMERCE, Frederick B. Dent, Secretary

NATIONAL BUREAU OF STANDARDS, Richard W. Roberts, Director

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# REFERENCE MATERIALS FOR THE DETERMINATION OF TRACE ELEMENTS IN BIOLOGICAL FLUIDS


## ABSTRACT

The preparation of a number of reference materials for the analysis of trace elements in biological standards is described. The standards produced include mercury in urine at three concentration levels, five elements [Se, Cu, As, Ni, Cr] in freeze-dried urine at two levels, fluorine in freeze-dried urine at two levels, and lead in whole blood at two concentration levels. These reference materials have been analyzed for the element(s) of interest by one or more analytical techniques, and are supplied with known concentration levels.


## 1. INTRODUCTION

The National Bureau of Standards (NBS) has prepared a number of working standards for the determination of trace metals and fluoride in biological fluids. The samples were prepared for the National Institute for Occupational Safety and Health (NIOSH) for use in methods evaluation and related investigations. The development and analytical approach used for these standards is described.

## 2. MERCURY IN URINE REFERENCE MATERIALS


T. E. Gills


### 2.1 Introduction

Urine is the biological fluid most often analyzed in cases of possible inorganic mercury exposure. For organo-mercurial exposure, serum is probably superior to urine since it gives a better measure of the body burden. The serum concentration of inorganic mercury, however, drops rapidly after the exposure; the kidney concentration stays elevated for some time after the exposure.

The original plan was to prepare the mercury in the urine standard by feeding mercury to experimental animals and collecting the urine, either by catheterization or in a metabolism cage. Through the cooperation of Dr. Eugene Miller at the U. S. Food and Drug Toxicology Laboratory in Beltsville, Maryland, a number of "minipig" laboratory pigs were fed inorganic mercury (as the nitrate). The samples were taken in a metabolism cage with a small plastic bag tied to the animal to minimize contamination from feces. The samples were filtered and acidified. Unfortunately, the mercury concentration of the porcine urine was only slightly above the normal background concentration. After conversations with toxicologists, it was determined that most, if not all, of the mercury in urine is inorganic and is probably unbound or bound very loosely. Because of these considerations, we decided that direct spiking of a urine sample would not be substantially different from a sample in which the mercury was present from a metabolic process.

A number of experiments were performed to determine the storage stability of mercury in urine. This was done by adding $^{203}Hg$ tracer to some samples of urine to which various amounts of acid had been added. It was found that most of the mercury would accompany the precipitate which formed on standing in non-acidified urine. If $\sim$5% of either $HNO_3$ or HCl were added to the urine, however, the mercury concentration was stable over several months.

Concurrently, experiments were performed to determine the feasibility of lyophilizing the urine as an alternate storage method. Several problems became obvious. The

mercury losses were higher than desired (>10%) and were not constant. It would, therefore, have been impossible to guarantee homogeneity from sample to sample. It was decided on the basis of these experiments to provide the urine as an acidified liquid for which the elevated concentrations were obtained by direct spiking.

## 2.2 Experimental

Eighteen liters of human urine were collected at NBS to be used as the mercury in urine standard. Six liters provided a standard at the "normal" concentration. Another six liters were doped to contain ∿90 mg Hg/l. The last six liters were doped to contain ∿200-250 mg/l.

The urine was collected from NBS personnel and stored in 6 liter Erlenmeyer flasks. Mercuric nitrate was used as a spike for the elevated levels. All of the standards were made 5% with high-purity nitric acid and stirred for 6 hours with a magnetic stirring bar. This acidity was chosen to prevent mercury adsorption on the surface of the flask. After mixing, 50 ml aliquots of the urine were decanted into 125-ml glass bottles. The bottles have Teflon-lined caps. The bottles of each standard were numbered sequentially so as to obtain information on the homogeneity of each lot.

After the preparation and bottling procedure, it was necessary to determine the homogeneity of both the elevated and normal levels by analyzing for the mercury content in each lot. Every tenth bottle was chosen for the homogeneity determination.

One milliliter aliquots were taken from each bottle and encapsulated in precleaned quartz vials. The samples, along with solution standards of mercuric nitrate, were irradiated for 1 hour in RT-3 of the NBS Reactor at a thermal neutron flux of $5 \times 10^{13} n \cdot cm^{-2} s^{-1}$. After allowing the activity to decay for 1-1/2 days to minimize personnel exposures, the ampoules were frozen in liquid nitrogen, broken, and the sample solution allowed to warm to room temperature. The solution was weighed and transferred, along with mercury carrier, to 125-ml Erlenmeyer flasks. Each sample was then digested with a 1:2 mixture of nitric acid and hydrochloric acid. After digestion a sulfide precipitation was performed and the mercuric sulfide was filtered onto glass-fiber filter pads.

Mercury-197 produced by the thermal neutron bombardment of mercury-196 was used as the measuring nuclide. The counting was done on a Ge(Li) low-energy photon detector in conjunction with a 1024 channel pulse height analyzer. The concentration was determined by comparing the activity in the samples to that of the standard.

## 2.3 Results and Discussion

The results for the elevated concentrations are given in Tables 1 and 2. For the "normal" urine samples, a concentration of <0.005 µg/g was found.

As an indication of the stability of these reference materials, the .084 mg/l samples were analyzed 6 months after preparation and the amount found was in total agreement with the first analysis.

TABLE 1

Mercury in Urine - "Elevated" Sample Number 1

| Bottle No. | mg/l | Ave. mg/l |
|---|---|---|
| 1 | .090<br>.092 | .091 |
| 10 | .087<br>.084 | .085 |
| 20 | .082<br>.084 | .083 |
| 30 | .081<br>.080 | .080 |
| 40 | .089<br>.087 | .088 |
| 50 | .080<br>.087 | .083 |
| 60 | .086<br>.088 | .087 |
| 70 | .087<br>.092 | .089 |
| 80 | .076<br>.072 | .074 |
| 90 | .090<br>.089 | .089 |
| | | Average = .085 |
| | | s = .005 |

TABLE 2

Mercury in Urine - "Elevated" Sample Number 2

| Bottle Number | mg/l |
|---|---|
| 30 | .216 |
| 10 | .216; .218 |
| 20 | .218 |
| 40 | .222 |
| 1 | .213 |
| 90 | .215 |
| 50 | .209 |
| 80 | .205 |
| 100 | .208 |
| 60 | .225 |
| 110 | .210 |
| | Average = .214 |
| | s = .006 |

## 3. SELECTED TRACE METALS IN FREEZE-DRIED URINE

L. T. McClendon, E. J. Maienthal, T. E. Gills

### 3.1 Introduction

The freeze-dried urine samples requested by the National Institutefor Occupational Safety and Health, consisting of a normal lot, a doped fluoride lot and a doped trace metal lot [Se, Cu, As, Ni, Cr(VI)], were obtained from the Lederle Laboratories and analyzed in the Analytical Chemistry Division of the National Bureau of Standards.

### 3.2 Experimental

The fluoride concentration of the urine samples was determined with the fluoride specific-ion electrode and will

be discussed separately below. The trace-metal concentrations were determined in both the normal lot and doped lot by the neutron activation analysis technique. Nickel and copper were also determined polarographically utilizing a sodium diethyl-dithiocarbamate extraction procedure to separate the copper and nickel from the matrix and measured polarographically in a pyridine-pyridinium sulfate supporting electrolyte.

In order to determine the trace metal concentrations in the freeze-dried urine by neutron activation analysis, radiochemical separations had to be performed because of the matrix radio-activity created in the urine samples during the irradiation. The procedure used for the determination of selenium, arsenic, and copper in the freeze-dried urine samples was as follows: The urine sample was reconstituted in 1 ml high-purity $HNO_3$ plus 10-ml ultra-pure $H_2O$ (11 ml total), instead of 50 ml $H_2O$ as prescribed by the suppliers, to obtain optimum sensitivity for the elements of interest. The concentrations found for the trace metals were corrected for a 50-ml dilution. Aliquots of the reconstituted urine sample, along with standards, were encapsulated in quartz vials, irradiated in the NBS Reactor at a flux of $\sim 1 \times 10^{13} n \cdot cm^{-2} \cdot s^{-1}$ for 30-60 minutes and allowed to decay for 8 hours to reduce short-lived interferences. For arsenic, copper, and selenium determinations, the irradiated urine samples were weighed, placed in 100-ml beakers, appropriate carriers added, and a mixture of $HClO_4$-$HNO_3$ added. The solutions were then heated to near dryness. After cooling the samples, HCl and KI were added and samples heated to reduce $As^{+5}$ to $As^{+3}$. The sulfides of As, Se, and Cu were then precipitated by the addition of a 5% thioacetamide solution. After filtration of each sample onto a 2.5 cm glass fiber filter pad, the samples

were counted on a high resolution Ge(Li) detector in conjunction with a 2048 channel pulse height analyzer.

The nickel concentration in the irradiated urine samples was determined by precipitation of nickel dimethylglyoximate (after carrier addition and pH adjustment), with filtration and counting of the precipitate as described previously. The chromium concentration in the irradiated urine samples was determined by the solvent extraction of the Cr(VI) tribenzylamine complex into chloroform with subsequent counting of an aliquot of the organic phase on the detector previously mentioned. It should be noted that chromium is present as Cr(III) instead of Cr(VI) in the freeze-dried urine samples as the technique used to determine chromium allows distinction of the two oxidation states. The commercial supplier assured us that chromium was indeed added as Cr(VI) (dichromate), but it is apparently reduced to Cr(III) in subsequent manipulations of the freeze-drying process.

### 3.3 Results and Discussion

The results of the trace metal analyses of the freeze-dried urine samples are shown in Tables 3, 4, and 5. The copper concentrations as shown in Table 4 are higher than specified due to the relatively high normal-copper values in the freeze-dried urine (Table 3). The difference in elevated nickel values between activation analysis (ave.=1.01 mg/l) and polarography (ave.=0.82 mg/l) is not believed to be significant in view of the estimated overall uncertainties of ±10 percent for each method. Accordingly, a recommended value for nickel of 0.9 mg/l ±0.1 is consistent with both measurements.

TABLE 3

Metals in "Normal" Freeze-Dried Urine
by Neutron Activation Analysis

| Metal | mg/l | Average (mg/l) |
|---|---|---|
| Cu | 0.028<br>0.029 | 0.028 |
| As | 0.022<br>0.023<br>0.025 | 0.023 |
| Se | 0.010<br>0.011 | 0.010 |
| Ni | <0.100 | <0.100 |
| Cr | 0.0261<br>0.0257 | 0.026 |

The samples as supplied to the National Institute for Occupational Safety and Health are labeled as Freeze-Dried Urine - "Normal" (285 total); "w/Fluoride" (95 total); "w/Metals" (84 total). The samples should be reconstituted with 50 ml of quartz-distilled water and are guaranteed to be as stable as fresh urine after reconstitution. The urine samples may be reconstituted with volumes of distilled water less than 50 ml if it is desired to have a solution more concentrated with the trace metals; however, the 50 ml volume is recommended. The urine samples should be refrigerated at all times.

TABLE 4

Metals in "Elevated" Freeze-Dried Urine
by Neutron Activation Analysis

| Metal | mg/l | Average mg/l | Specified Concentrations mg/l |
|---|---|---|---|
| Cu | 0.068 | 0.062 | 0.030 |
| | 0.061 | | |
| | 0.067 | | |
| | 0.063 | | |
| | 0.060 | | |
| | 0.054 | | |
| | 0.060 | | |
| As | 0.98 | 0.96 | 1.00 |
| | 0.99 | | |
| | 0.91 | | |
| Se | 0.027 | 0.026 | 0.025 |
| | 0.025 | | |
| Ni | 1.15 | 1.01 | 1.00 |
| | 0.92 | | |
| | 0.93 | | |
| | 1.06 | | |
| Cr | 0.047 | 0.047 | 0.050 |
| | 0.051 | | |
| | 0.046 | | |
| | 0.043 | | |

TABLE 5

Metals in Freeze-Dried Urine by Polarography

| Metal | Concentrations (mg/l) Normal | Elevated |
|---|---|---|
| Cu | 0.026<br>0.026<br>0.026 average | 0.0608<br>0.0626<br>0.0617 average |
| Ni | ≤0.02 | 0.81<br>0.84<br>0.82 average[a] |

[a]Estimated uncertainty = ±10%

## 4. FLUORINE IN FREEZE-DRIED URINE

R. A. Durst

### 4.1 Introduction

The determination was carried out by the method of standard additions, using a Gran plot extrapolation to the end point. A combination fluoride electrode was used to monitor fluoride concentration during the analysis.

### 4.2 Experimental

The procedure consisted of diluting 10 ml of the reconstituted urine with 10 ml of a total ion strength adjustment buffer (TISAB) and adding three aliquots of a standard fluoride solution. The TISAB (57 ml/l glacial acetic acid, 58 g/l NaCl, 0.3 g/l sodium citrate and adjusted to pH 5.0-5.5 with NaOH) was used to maintain the ionic strength and pH constant. The standard fluoride solution contained 0.306 g KF/l for a fluoride concentration of 0.1 mg $F^-$/ml.

## 4.3 Results and Discussion

For the urine samples with elevated fluoride, nominally 7 mg/l (ppm), three 1 ml aliquots of the fluoride standard were used and the data were plotted on 10 percent volume-corrected Gran paper. Five samples were run in duplicate and the results are given in Table 6. An average of 7.8 ± 0.1 mg $F^-$/l was found with no indication of inhomogeneity between samples.

The normal urine samples were spiked with three 100 µl aliquots of the standard fluoride solution and the data were plotted on zero volume-corrected Gran paper. Three samples were run in duplicate and the results are given in Table 6. An average of 0.41 ± 0.02 mg $F^-$/l was found.

A duplicate blank determination on the TISAB gave a value of 0.01 mg $F^-$/l. The above values and the data in Table 6 are not corrected for this small blank.

The freeze-dried urine samples should be handled as specified in section 3.3.

TABLE 6

Fluoride Concentration in Freeze-Dried Urine

| "Elevated" Fluoride | mg/l |
|---|---|
| Sample 1 | 7.9 |
| | 7.9 |
| Sample 2 | 7.8 |
| | 7.7 |
| Sample 3 | 7.7 |
| | 7.8 |
| Sample 4 | 7.7 |
| | 7.7 |
| Sample 5 | 7.7 |
| | 7.7 |
| "Normal" Fluoride | mg/l |
| Sample 1 | 0.39 |
| | 0.42 |
| Sample 2 | 0.41 |
| | 0.40 |
| Sample 3 | 0.43 |
| | 0.42 |
| Blank | mg/l |
| Sample 1 | 0.01 |
| Sample 2 | 0.01 |

# 5. LEAD IN BLOOD REFERENCE MATERIAL

L. T. McClendon, J. W. Gramlich, L. A. Machlan,
T. J. Murphy, E. J. Maienthal, D. A. Becker

## 5.1 Introduction

The determination of lead in blood is of interest both to occupational health laboratories and clinical laboratories. Because of the importance of this analysis in inner-city areas for the determination of lead in blood of children, considerable work has been completed in the areas of analytical technology and sample preparation. As a result of the considerable effort for these samples, much information has been obtained.

Most workers in the field feel that for the analysis of the blood samples containing elevated lead concentrations, the lead should be present from normal metabolic processes, as opposed to a direct spiking technique. For this reason, the elevated lead in blood samples prepared for the National Institute for Occupational Safety and Health through the National Bureau of Standards, were obtained by feeding lead acetate to pigs. This feeding was accomplished through the courtesy of Dr. E. Miller of the United States Food and Drug Administration Toxicology Laboratory.

## 5.2 Experimental

After a several-week feeding regimen, the pigs were sacrificed and blood samples were obtained by way of heart puncture on the animals. Approximately 1.5 liters of blood from these animals were obtained. In this particular case the scientists in the FDA laboratory used ethylenediaminetetraacetic acid (EDTA) as the anticoagulent. A second two-liter sample of pig blood was obtained from animals which had not been fed lead other than that from normal dietary sources. For this blood, sodium heparin was used as the anticoagulent.

Upon arrival at NBS, samples were taken of the two blood sources and were analyzed polarographically for confirmation of proper approximate concentrations of the starting material and to detect any changes in the lead concentration that might occur in the processing sequences. In preparing the material for use as a standard it was desired to have material which was as homogeneous as possible and which had been hemolyzed. To hemolyze the blood it was frozen at a temperature of -12 °C for 24 hours, thawed and refrozen and rethawed twice at the same temperatures and times. After this treatment some cryoproteins had apparently been precipitated as two phases were obviously present in the sample.

The samples were then treated with an ultrasonic cell disrupter to break up as many of these remaining unlysed cells as possible. After sonification, the blood samples were centrifuged, and the supernatant liquid and precipitate were then analyzed by polarographic techniques. At this point it was demonstrated that the lead concentration in the liquid phase was essentially the same as the starting material, with only a minute amount of the lead in the solid phase. After centrifugation, all of the supernate liquid was combined and mixed well. All of the operations with both the high concentration and low concentration blood samples were carried out in preleached (with high purity $HNO_3$) and presterilized Teflon or polypropylene containers.

## 5.3 Results and Discussion

After careful mixing, the blood samples were packaged in 12-ml sterile polycarbonate sample containers and random samples were picked for assay by isotope dilution mass spectroscopy. Seven ampoules of the blood were

analyzed for this purpose; five ampoules of blood at the elevated concentration and two background concentration samples. Each ampoule of elevated blood was sampled with one five-gram and two three-tenths-gram aliquots; for the background blood, only five-gram aliquots were used. The results of these analyses are shown in Tables 7 and 8. Three analytical blank determinations were made which averaged 5.5 nanograms of lead. This gave a blank contribution of approximately 0.1%, 1.6%, and 2.9% to the five gram aliquots, the three-tenths-gram elevated blood aliquots and the five-gram background aliquots, respectively.

After shipment to NIOSH of the lead-in-blood samples, it became obvious that the normal levels for porcine blood were too low to be easily determined. Therefore, a second set of normal levels was produced, from mixed normal and elevated levels of porcine blood. This mixture was thoroughly agitated and homogenized, then packaged and delivered under conditions similar to the earlier samples. The analyses of this second set of normal samples are found in Table 9.

The samples as supplied to the National Institute for Occupational Safety and Health are in ∿12 gram polycarbonate sample containers labeled as either high level or low level lead. We are satisfied as to their homogeneity and the concentration value of the lead obtained using isotope dilution mass spectroscopy. This is an absolute technique demonstrated to have an accuracy of better than 0.5% absolute. Prior to analysis, the sample should be shaken vigorously for 60 seconds and, if desired, may be treated by sonification. The samples should be refrigerated at all times.

TABLE 7

Elevated Lead Concentration in Porcine Blood

| Vial Number | Aliquot Number[a] | ppm Pb[a] | ppm Pb[b] |
|---|---|---|---|
| 1 | 2 | 1.021 | 1.042 |
| | 3 | 1.036 | 1.042 |
| 18 | 2 | 0.987 | 0.992 |
| | 3 | 0.985 | |
| 33 | 2 | 1.001 | 0.990 |
| | 3 | 0.985 | |
| 47 | 2 | 0.995 | 0.992 |
| | 3 | 0.990 | |
| 61 | 2 | 0.986 | 0.990 |
| | 3 | 0.988 | |
| | Average = | 0.997 | 1.001 |
| | s = | 0.017 | 0.023 |

[a]Sample size = 0.300 g
[b]Sample size = 5.00 g

TABLE 8

Normal Lead Concentrations in Porcine Blood

| Vial Number | ppm Pb[a] |
|---|---|
| B22 | 0.0335 |
| B117 | 0.0294 |
| Average = | 0.0315 |

[a]Sample size = 5.00 g

TABLE 9

"Elevated Normal" Lead Concentration in Porcine Blood

| Sample | Aliquot | ppm Pb |
|---|---|---|
| 5 | 1 | 0.1438 |
| | 2 | 0.1436 |
| 6 | 1 | 0.1367 |
| | 2 | 0.1352 |

## 6. CONCLUSION

The various reference materials produced and supplied, along with known concentration levels for the elements of interest, should be extremely useful for the evaluation of field and laboratory analytical methods for the analysis of toxic elements. In particular, the use of at least two calibration points (i.e., "normal" and "elevated" levels) for a given matrix should provide a more positive calibration over the range of interest for occupational toxicological levels of exposure.

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The preparation of a number of reference materials for the analysis of trace elements in biological standards is described. The standards produced include mercury in urine at three concentration levels, five elements [Se, Cu, As, Ni, Cr] in freeze-dried urine at two levels, fluorine in freeze-dried urine at two levels, and lead in whole blood at two concentration levels. These reference materials have been analyzed for the element(s) of interest by one or more analytical techniques, and are supplied with known concentration levels.

17. KEY WORDS (*six to twelve entries; alphabetical order, capitalize only the first letter of the first key word unless a proper name; separated by semicolons*) Arsenic; biological fluids; chromium; copper; fluorine; lead; nickel; reference materials; selenium; trace elements; urine.

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