M.W.: Table 1 FORMULA: Table 1 CAS: Table 1 RTECS: Table 1

METHOD: 8005, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 May 1985**
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BIOLOGICAL INDICTOR OF: exposure to the following elements or their compounds: antimony, cadmium, chromium, cobalt, copper, iron, lanthanum, lead, lithium, magnesium, manganese, molybdenum, nickel, platinum, silver, strontium, thallium, vanadium, zinc and zirconium.

SYNONYMS: vary according to compound.

BIOLOGICAL SAMPLING		MEASUREMENT	
SPECIMEN:	BLOOD OR TISSUE	**METHOD:**	INDUCTIVELY-COUPLED ARGON PLASMA-ATOMIC EMISSION SPECTROSCOPY (ICP-AES)
VOLUME:	10 mL (blood) or 1 g (tissue)	**ANALYTE:**	elements above
PRESERVATIVE:	heparin (blood); none for tissue	**DIGESTION ACID:**	3:1:1 (v/v/v) HNO_3:$HClO_4$:H_2SO_4
SHIPMENT:	frozen for blood and "wet" tissue; routine for "dry" tissue	**FINAL SOLUTION:**	10% H_2SO_4; 10 mL (blood), 5 mL (tissue)
STABILITY:	not established	**WAVELENGTH:**	varies with element; Table 2
CONTROLS:	collect at least 3 blood specimens from unexposed workers	**BACKGROUND CORRECTION:**	spectral wavelength shift
		CALIBRATION:	elements in 10% H_2SO_4 or yttrium internal standard
		QUALITY CONTROL:	spiked blood or tissue; reference materials
		RANGE:	10 to 10,000 µg/100 g blood; 2 to 2000 µg/g tissue
		ESTIMATED LOD:	1 µg/100 g blood; 0.2 µg/g tissue
		PRECISION ($\bar{S}_r$):	Table 2
		ACCURACY:	Table 2

APPLICABILITY: This method is useful for monitoring the blood of workers exposed to several metals simultaneously. This is a simultaneous multielemental analysis, but is not compound-specific.

INTERFERENCES: Spectral interferences are sometimes encountered. These are minimized by judicious wavelength selection and interelement correction factors. Background corrections (spectral wavelength shift) are also made [1,2].

OTHER METHODS: This method uses a measurement technique similar to that of Methods 7300 (Elements; for air samples) and 8310 (Metals in urine).

REAGENTS:

1. Nitric acid, conc. (HNO_3) (high purity).*
2. Perchloric acid, conc. ($HClO_4$) (high purity).*
3. Sulfuric acid, conc. (H_2SO_4) (high purity).*
4. Digestion acid, 3:1:1 (v/v/v) HNO_3:$HClO_4$:H_2SO_4.* Mix three volumes conc. HNO_3 with one volume conc. $HClO_4$ and one volume conc. H_2SO_4.
5. Element standards, 1000 µg/mL. Commercially available or prepared per instrument manufacturer's recommendations.
6. Reference materials of known elemental composition. SRM #1577a, Bovine Liver, from the U.S. National Institute of Standards and Technology is recommended.
7. Argon.
8. Deionized water.
9. Yttrium standard, 5 µg/mL in 5% HNO_3. Combine 50 mL conc. HNO_3, ca. 500 mL deionized water, and 5 mL 1000 µg Y/mL standard. Dilute to 1 L.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Blood collection tubes, heparinized, lead-free, specially prepared for collecting blood samples for blood lead determinations.
   NOTE: Heparin may contain significant amounts of Ca, Cu, Mn, Sr and Zn and should not be used if these elements are to be determined [3].
2. Vacutainer needles (21-gauge) and holder.
   NOTE: These may be significant sources of contamination in some cases [4].
3. Tourniquet and alcohol swabs.
4. Bottles, glass or polyethylene, with PTFE-lined caps, 20-mL (e.g., scintillation vials).*
5. Inductively-coupled plasma-atomic emission spectrometer equipped for determination of elements of interest.
6. Regulator, two-stage, for argon.
7. Analytical balance, readable to 1 mg.
8. Beaker, Griffin (50-mL) or Phillips (125-mL), with watchglass covers.*
9. Hotplate, for use at 110 and 250 °C.
10. Pipets, 5- and 10-mL, with pipet bulb.*
11. Volumetric flasks, 5- and 10-mL and 1-L.*
12. Plastic,* glass* or single element (e.g., tantalum) knives and forceps for cutting tissue samples, as needed [3,4,5].
13. Work station with plastic work surfaces, with air cleaned by a vertical, laminar flow device and high efficiency particulate air filter or by electrostatic precipitator.
14. Gloves, plastic, metal-free. *All glassware and plasticware which contacts standards, blanks, or samples should be detergent-washed, thoroughly rinsed with tap and deionized water, soaked 12 h in 10% (v/v) HNO_3 and soaked 12 h in deionized water.

SPECIAL PRECAUTIONS: Concentrated acids are extremely corrosive; work with them only in a fume hood and wear appropriate safety equipment (safety glasses or face shield, gloves and labcoat).

Samples of blood and tissue collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle blood and tissue specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided.

SAMPLING:

1. Collect blood samples in heparinized blood collection tubes. Mix immediately. Collect tissue samples (ca. 0.25 g for "dry" tissue or ca. 1 g for "wet" tissue) in bottles.
   NOTE: If unheparinized blood collection tubes are used, freeze samples immediately.

2. Ship samples in dry ice and store frozen (<15 °C) prior to digestion.

SAMPLE PREPARATION:

3. Allow sample to equilibrate to room temperature.
4. Transfer accurately weighed portions of 10 g blood, 0.25 g "dry" tissue, or 1.0 g "wet" tissue to a beaker.
5. Add 10.0 mL digestion acid to each blood sample or 5.0 mL digestion acid to each tissue sample. Heat at 110 °C for 2 h.
   NOTE: Start reagent blanks, in triplicate, at this step.
6. Increase hotplate temperature to 250 °C and heat until ca. 1 mL (for blood) or ca. 0.5 mL (for tissue) remains (2 to 3 h).
7. Allow beaker to cool.
8. Choose one of the following:
   a. **External standard method.** Transfer contents of beaker to a volumetric flask (10 mL for blood; 5 mL for tissue). Dilute to the mark with deionized water.
   b. **Internal standard method.** Add, via pipet, 10.0 mL (for blood) or 5.0 mL (for tissue) yttrium standard to the beaker.

CALIBRATION AND QUALITY CONTROL:

9. Calibrate the spectrometer according to manufacturer's recommendations.
   NOTE: Typically, an acid blank and 10 µg/mL multielement solutions are used.
10. Analyze a standard for every ten samples.
11. Check measurement recoveries for all elements of interest with at least three spiked, unexposed samples or with reference materials of known elemental composition. These quality control samples should constitute 15 to 20% of all samples analyzed.
    NOTE: For blood or tissue spikes, split a control sample and spike one fraction. Subtract the element quantity found in the unspiked portion from the element quantity found in the spiked portion and determine measurement recovery. Correct all samples for this measurement recovery. This is especially important for blood samples because routine interelement corrections will not adequately compensate for the high levels of iron present, which has numerous spectral emission wavelengths.

MEASUREMENT:

12. Set the spectrometer to conditions specified by the manufacturer.
13. Analyze standards and samples.
    NOTE: If the values for the samples are above the range of the standards, dilute the sample solutions with 10% H_2SO_4, reanalyze, and apply the appropriate dilution factor in calculations.

CALCULATIONS:

14. Obtain the solution concentration found for each element in the sample, C_s (µg/mL), and the average blank, C_b (µg/mL), from the measurement data.
15. According to the method of standardization, calculate the concentration, C (µg/g), of each element in the mass of sample taken, M (g).
    a. **External standard method.** Use the final solution volumes of sample, V_s (mL) and blank, V_b (mL):
    b. **Internal standard method.** Use the yttrium standard concentration, C_y (µg/mL), the volume of yttrium standard added, V_y (mL), and the concentration of yttrium found in the

$$C = \frac{(C_s V_s - C_b V_b)}{M}, \mu g/g.$$

sample, C_{sy} (µg/mL). Treat the blank solution similarly [i.e., C_b is the concentration (µg/mL) of analyte on the blank and C_{by} is the concentration of yttrium in the blank]:

$$C = \frac{\frac{C_s C_y V_y}{C_{sy}} - \frac{C_b C_y V_y}{C_{by}}}{M}, \mu g/g.$$

GUIDELINES TO INTERPRETATION:

Acceptable and unacceptable levels for elements have not been determined by this method. Iyengar [6] reports metals concentrations in tissues and body fluids. Lauwerys [7] discusses metals and can be consulted for guidance and interpretation. For tissue, trace element concentrations will vary with the tissue type or organ. Iyengar [6] reports metals concentrations for different tissues of non-occupationally exposed people. Blood metal concentrations of non-occupationally exposed individuals are given in Table 2.

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TABLE 1. GENERAL INFORMATION

Element (Formula)	Atomic Weight	CAS#	RTECS
Antimony (Sb)	121.75	7440-36-0	CC4025000
Cadmium (Cd)	112.40	7440-43-9	EU9800000
Cobalt (Co)	58.93	7440-48-4	GF8750000
Chromium (Cr)	52.00	7440-47-3	GB4200000
Copper (Cu)	63.54	7440-50-8	GL5325000
Iron (Fe)	55.85	7439-89-6	NO4565500
Lanthanum (La)	138.91	7439-91-0	--
Lead (Pb)	207.19	7439-92-1	OF7525000
Lithium (Li)	6.94	7439-93-2	OJ5540000
Magnesium (Mg)	24.31	7439-95-4	OM2100000
Manganese (Mn)	54.94	7439-96-5	OO9275000
Molybdenum (Mo)	95.94	7439-98-7	QA4680000
Nickel (Ni)	58.71	7440-02-0	QR5950000
Platinum (Pt)	195.09	7440-06-4	TP2160000
Silver (Ag)	107.87	7440-22-4	VW3500000
Strontium (Sr)	87.62	7440-24-6	--
Thallium (Tl)	204.37	7440-28-0	XG3425000
Vanadium (V)	50.94	7440-62-2	YW1355000
Zinc (Zn)	65.37	7440-66-6	ZG8600000
Zirconium (Zr)	91.22	7440-67-7	ZH7070000

TABLE 2. RECOVERY OF METALS FROM BLOOD [1,2].

Element (Formula)	Wavelength (nm)	Value[a] (µg/100 mL)	Metal "Nonexposed" Added (µg/Sample)	Quantity Recovery (%)	Precision (% s_r) n = 4	Accuracy (±%)
Antimony (Sb)	217.58	0.4	10	106	4.9	15.6
Cadmium (Cd)	226.5	0.5	10	120	1.1	22.2
Cobalt (Co)	231.2	1.0	10	81	21	60.2
Chromium (Cr)	205.6	4.5	10	114	4.7	23.2
Copper (Cu)	324.8	100	10	101	5.8	12.4
Iron (Fe)	45,000	0	--[b]	--[b]		--
Lanthanum (La)	--[c]	10	119	2.4		23.7
Lead (Pb)	220.4	23	10	113	0.85	14.7
Lithium (Li)	670.8	1.0	10	113	1.1	15.2
Magnesium (Mg)	279.6	3,800	110	104	12	27.5
Manganese (Mn)	257.6	4.0	10	98	2.1	6.1
Molybdenum (Mo)	281.6	4.0	10	126	3.1	32.1
Nickel (Ni)	231.6	5.0	10	86	16	45.4
Platinum (Pt)	203.7	--[c]	10	92	14	35.4
Silver (Ag)	328.3	3.5	10	115	0.8	16.6
Strontium (Sr)	421.5	2.8	10	113	0.88	14.7
Thallium (Tl)	190.9	1.0	10	97	8.7	20.0
Vanadium (V)	310.2	1.2	10	131	1.1	33.2
Zinc (Zn)	213.9	700	60	103	17	36.3
Zirconium (Zr)	339.2	1.5	10	71	8.7	46.0

[a]"Nonexposed" value is the average concentration for the respective element in blood of non-occupationally exposed individuals. These values were tabulated from References [6] through [19].

[b]Recovery not determined (blood Fe concentration was above quantitation limit of spectrometer).

[c]Concentration not reported.