LEAD in blood and urine 8003

Pb MW: 207.19 CAS: 7439-92-1 RTECS: OF7525000

METHOD: 8003, Issue 2	EVALUATION: FULL	Issue 1: 15 February 1984 Issue 2: 15 August 1994

BIOLOGICAL INDICATOR OF: exposure to lead and lead compounds.

SYNONYMS: none.

BIOLOGICAL SAMPLING		MEASUREMENT	
SPECIMEN:	whole blood; urine, end of shift	**TECHNIQUE:**	ATOMIC ABSORPTION, FLAME
CONTAINER:	10-mL lead-free tubes (blood); 125-mL polyethylene bottles (urine)	**ANALYTE:**	Pb(II)-APDC complex
ANICOAGULANT:	(blood) heparin	**EXTRACTION:**	APDC-MIBK
PRESERVATIVE:	(urine) 0.2 mL conc. HNO_3	**CALIBRATION:**	methyl isobutyl ketone solutions of Pb(II)-APDC complex
SHIPMENT:	polyethylene shippers	**QUALITY CONTROL:**	commercial controls; pooled urine or blood; urine corrected for creatinine
SAMPLE STABILITY:	blood stable 3 days @ 4 °C; indefinitely if sonicated and frozen in plastic tubes; urine stable indefinitely if kept acidified with HNO_3	**RANGE:**	5 to 150 µg/100 g blood; 5 to 150 µg/100 mL urine
		ESTIMATED LOD:	0.05 µg Pb/g blood or mL urine [1]
CONTROLS:	commercial urine and blood lead control samples plus pooled urine and blood from non-exposed populations	**RECOVERY:**	0.99
		PRECISION ($\bar{S}_r$):	0.05 [1]
		ACCURACY:	± 10.8%

APPLICABILITY: This procedure quantitates Pb^{2+} in blood or urine to assess body burden, injury to the hematopoietic system, and to comply with Federal Regulations. Blood lead is the preferred biological indicator of lead absorption. The optimum working range is 0.1 to 1.5 µg. Pb per g blood or per mL urine [2].

INTERFERENCES: Phosphate, EDTA, and oxalate can sequester lead and cause low lead readings.

OTHER METHODS: This method combines and replaces P&CAM 208 [1] and 262 [3]. Methods P&CAM 102 (dithizone), 195 (ASV), 200 (ASV) and 214 (graphite furnace AAS) [4] have not been revised. Anodic stripping voltametry (ASV) is an alternative procedure if specialized equipment is available [5].

REAGENTS:

1. Ammonium pyrrolidine dithiocarbamate (APDC). Stable 6 months.
2. Nonionic surfactant, octyl phenoxy polyethoxy ethanol (Triton X-100 or equivalent).
3. APDC-surfactant solution (APDC-Tx). Dissolve 4 g APDC and 5 mL nonionic surfactant in 40 mL deionized water. Dilute to 200 mL. Check each batch for extraction efficiency. Stable at least two months.
4. Lead nitrate $Pb(NO_3)_2$. Heat 4 h at 120 °C. Cool and store in dessicator.
5. Nitric acid, conc. 70% (w/w), redistilled.
6. Methyl isobutyl ketone (MIBK), water-saturated. Add 100 mL water to 900 mL MIBK. Shake and allow to stand 1 h.
7. Calibration stock solution (1000 µg/mL). Dissolve 1.598 g $Pb(NO_3)_2$ in 2% (w/v) HNO_3 and dilute to 1 L. Store in a polyethylene bottle. Stable 1 year.
8. Air at 40 psi, filtered to remove oil and water.
9. Acetylene, grade recommended by AAS manufacturers.

EQUIPMENT:

1. Heparinized, lead-free, "blue-top" blood collection tubes specially prepared for collecting blood samples for blood lead determinations.
2. Vacutainer needles (21-gauge) and holder.
3. Tourniquet and alcohol swabs.
4. Polyethylene bottles (wide mouth), 125-mL.
5. Culture tubes, 16 x 100-mm, with PTFE-lined screw caps.*
6. Centrifuge.
7. Rotary vibration mixer (Vortex) or equivalent.
8. Atomic absorption spectrophotometer (AAS) with lead hollow cathode or electrodeless discharge lamp.
9. Analytical balance.
10. Desiccator.
11. Pipettes, volumetric flasks and other appropriate glass or plasticware for weighing, storing, and dispensing reagents and samples.*

* All glassware and plasticware should be detergent washed, thoroughly rinsed with tap and deionized water, soaked 4 hrs in 1:1 HNO_3:H_2O and finally thoroughly rinsed with deionized water.

SPECIAL PRECAUTIONS: Samples of blood and urine 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 urine 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. Ship at 4 °C and maintain at this temperature prior to analysis.
2. Collect urine at the end of a work shift in 125-mL polyethylene bottles containing 0.2 mL conc. HNO_3; mix. Submit a 25-mL aliquot for lead analysis and another 25-mL aliquot for creatinine determination.

SAMPLE PREPARATION:

3. Filter urine samples before analysis. Analyze a 25-mL aliquot for creatinine by standard procedures (e.g., [6]).
4. Place 2.0 mL filtered urine or 2.0 g whole blood in a 16 x 150-mm culture tube. Start a reagent blank at this point with 2 mL deionized water.
5. Add 0.8 mL APDC-surfactant solution. Cap and mix on a rotary vibration mixer for 10 sec.
6. Add 2.00 mL water-saturated MIBK. Cap and rotate on a rotary vibration mixer for 2 min. Centrifuge at 2000 rpm for 10 min. Analyze the Pb-APDC solution in MIBK within 2 h of extraction.

NOTE: If the patient is receiving EDTA therapy or if blood specimens are collected in tubes containing Na_2 EDTA as anticoagulant, add 50 µL 1.5 M $CaCl_2$ immediately before adding the MIBK [7].

CALIBRATION AND QUALITY CONTROL:

7. Prepare 6 working standards in deionized water in the range 10 to 150 µg/100 mL Pb by dilution of calibration stock solution with 2% (w/v) HNO_3. Prepare fresh daily.
   Example: 0.4 mL calibration stock stolution diluted to 1 L = 40 µg per 100 mL.
8. Analyze the working standards (steps 4, 5, 6, 12, and 13).
9. Prepare a calibration graph. Plot concentration (µg/100 mL) vs. absorbance of working standard, corrected for reagent blank.
10. Maintain standardization by analyzing a standard after every 5 samples.
11. Run a spiked control or pooled sample from exposed workers or commercial control every 10 samples or at least 3 per study.
    NOTE: Blood or urine lead background levels must be determined before spiked controls are prepared.

MEASUREMENT:

12. Set the atomic absorption spectrophotometer and lamp conditions as recommended by the manufacturer and as given on page 8003-1. Aspirate water-saturated MIBK and reduce acetylene flow until a stoichiometric flame with minimum background is obtained (< 0.01 absorbance). Record this reading.
13. Aspirate each sample and blank. Record maximum absorbances.
    NOTE: Samples with absorbance values greater than 0.35 must be diluted with MIBK and reanalyzed. Apply the appropriate dilution factor in calculations.

CALCULATIONS:

14. Calculate net absorbance (difference between the maximum absorbance of MIBK and the absorbance of the sample or blank).
15. Using metals, determine blood and urine lead levels in the samples (W_s) and average reagent blanks (B) from the calibration graph.
16. For urine samples, report µg Pb/g creatinine. For blood samples, report µg Pb/g whole blood.

GUIDES TO INTERPRETATION:

Blood lead concentrations for healthy adult suburbanites is 7 to 22 µg/100 g whole blood [2]. Urine has a wider range, 4 to 270 µg/g creatinine, for the same population; however, most normal values should be near 16 to 60 µg/g creatinine [4].

Blood lead values are commonly used for biological monitoring. A blood lead value above 40 µg/100 g is indicative of excess exposure and one above 60 µg/100 g requires removal from exposure [8].

EVALUATION OF METHOD:

Forty-two blood specimens were spiked at levels of 25 to 200 µg/100 mL. Recoveries were 94 to 106%, averaging 100% [7].

For urine, 25 specimens were spiked at concentrations from 10 to 100 µg/mL. Recoveries averaged 100% (range 95 to 106%) [7].

REFERENCES:

[1] NIOSH Manual of Analytical Methods, 2nd ed., V. 1, P&CAM 208, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A (1977).
[2] Baselt, R. C. Biological Monitoring Methods for Industrial Chemicals, p. 159, Biomedical Publications, Davis, CA (1980).
[3] NIOSH Manual of Analytical Methods, 2nd. ed., V. 1, P&CAM 262, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A (1977).
[4] Ibid., P&CAM 102, 195, 200, and 214.
[5] Environmental Sciences Associates, Inc. Methods Manual Methods TMA-1 and TMA-2, Bedford, MA.
[6] Hessel, D. W. Atomic Absorption Newslett., 7, 55 (1968).
[7] Zenterhofer, L. J. M., P. I. Jatlow, and A. Fappiano. Atomic Abosrption Determination of Lead in Blood and Urine in the Presence of EDTA, J. Lab. Clin. Med., 78, 664-674 (October, 1971).
[8] Occupational Safety and Health Administration, Chapter XVII, 1910.1025, Section C, 691.

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