Optimization of a GF-AAS method for lead testing in blood and urine: A useful tool in acute abdominal pain management in emergency

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Abstract. Suspicion of lead poisoning is confirmed by its concentration in blood and protoporphyrin red blood cells. At low concentrations, lead influences the synthesis of the heme in the sense of lowering it. Acute and chronic lead intoxication is extremely polymorphic in regards to its clinical manifestations, with digestive, hematological, cardiovascular, renal hepatic and neurological features. The aim of the study was to evaluate the presence of lead in human whole blood and urine harvested before and during chelation treatment in the case of lead poisoning. An atomic absorption spectroscopic method for the analysis of lead was developed using graphite furnace atomic absorption spectrophotometer (GF-AAS), Varian Spectra AA-880 with a hollow cathode lead lamp and a deuterium lamp for background correction, coupled to a GTA-100 atomizer and a programmable sample dispenser. Standard calibration solutions were used for the range 10-100 μg/l. The linearity range was 10.0 to 100.0 μg/l with the correlation coefficient of 0.999. We established that the method can be applied for the determination of lead in whole blood and urine, and the results obtained are useful for monitoring chelation therapy in cases of acute lead poisoning, a neglected cause of abdominal colic pain in an emergency situation.

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Abbreviations: GF-AAS, graphite furnace atomic absorption spectrometry

Key words: lead toxicity, GF-AAS, biological samples, saturnine colic, acute abdominal pain

Introduction

Lead intoxication is an environmental health problem, with extremely severe consequences upon the human body (1). Acute lead poisoning manifests clinically with intense acute abdominal pain, being a challenging diagnosis in emergency situations (2). Development a quick and reliable method for lead determination in blood and urine, is important in clinical practice, both in diagnostics, but also in monitoring chelating therapy.

The main sources of lead that result in lead contamination include: Paint; leaded petrol; drinking water; car batteries, cables, glass; printers with lead-based technology; manufacture and use of war ammunition (3,4). In melters, the main danger is melting. The risk of exposure to lead increases with increasing temperature in industrial processes (5).

The main routes of absorption of lead are gastric and pulmonary. Gastric absorption in adults is about 10-15% of the total amount ingested and 40% in children. At the lung level it is absorbed at ~50-70% of the inhaled dose. In organic form, lead from tetraethyl lead is also absorbed at the skin level (6). Inhalation absorption depends on the form of lead (vapors or particles). Approximately 90% of inhaled particulate lead is absorbed into the respiratory tract (7). Once absorbed, 99% is

transported by the bloodstream and binds to hemoglobin in red blood cells (2,5).

The distribution is completed in three compartments, namely in the blood compartment, mineralized tissues (bones, teeth) and soft tissues (kidneys, bone marrow, liver and brain). Lead in the blood is distributed at a percentage of 99% in erythrocytes and 1% in plasma and is available for transport to tissues. The blood concentration does not reflect the actual amount of lead in the body, but ~90% is stored in tissues, with a maximum half-life of ≤30 years (8). Because lead is mobilized from these tissues, individuals who have been exposed may have high concentrations of lead, from a few months to several years from the time of exposure cessation (7). The level of lead in the blood, a traditional indication of absorption, reflects only recent exposure as the half-life in the blood is 36 days (3). In individuals with chronic exposure, there is a small correlation between a level once determined at a control and the cumulative absorption index or loading of the lead organism.

Suspicion of lead poisoning is confirmed by its concentration in blood and protoporphyrin red blood cells. At low concentrations, lead influences the synthesis of the heme in the sense of lowering it. Due to the fact that the level of erythrocyte protoporphyrin is not pathognomonic in children at levels of approximately 25 μg/dl, the best method remains to determine the level of blood lead (9,10). Lead binds to the sulfur groups of many enzymes by inactivating them. Lead poisoning has a multisystemic, hematological, cardiovascular, renal, hepatic, digestive and neurotoxic impact (5). Acute intoxication at blood levels >50 μg/dl causes saturnine colic, and biochemical dosing of lead when there is clinical suspicion is extremely important in emergency situation, in the differential diagnosis of acute abdomen, to avoid white laparotomies in patients with severe abdominal colic pain (11).

This work aims to present a method of determination for lead in blood and urine using graphite furnace atomic absorption spectrometry (GF-AAS) with a background correction. Atomic absorption spectrometry with graphite furnace atomization (GF-AAS) is a leading technique in analytical chemistry as a routine low-level assay for lead and other heavy metals, for a wide variety of sample types (12).

Materials and methods

Instrumentation. Lead analysis was performed using a graphite furnace atomic absorption spectrophotometer (GF-AAS) Varian Spectra AA-880, with a hollow cathode lamp (Agilent Technologies, Inc.) and a deuterium lamp (Agilent Technologies, Inc.) for background correction, coupled to a GTA-100 atomizer and a programmable sample dispenser (Varian). Addition instrumentation included: A monochromator (fully automatic computer-controlled Czerny-Tuner micromotor, focal length 0.33 mm; automatic sample dispenser PSD Varian with 54 positions for samples, standards, modifiers, quality control and buffer, maximum injected quantity 100 μl, injection precision 0.2 μl, automatic dilution and mixing, automatic re-injection of samples); Neslab CFT 33 water cooler for graphite oven working at temperature 15-25˚C; a nitrogen generator (Dominik Hunter) (purity, 99.999%); EBA 200 Hettich Centrifuge, Eppendorf automatic pipette 1,000 μl. Biochemical parameters were determined from blood on the Vitros 650 System (Ortho Clinical Diagnostics) and complete blood count on a Celltac-F Hematology Analyzer (Nihon Kohden).

Table I. Working parameters for determining lead by GF-AAS.

No.	Parameter	Method
1.	Injection mode	Automated dilution
2.	Calibration mode	Concentration
3.	Type of measurement	Peak height
4.	Replicate standard	3
5.	Replicate sample	3
6.	Smoothing	9
7.	Wavelength	283.3 nm
8.	Slit	0.5
9.	Lamp current	10 mA
10.	Background correction	Yes
11.	Standard 1	10 μg/l
12.	Standard 2	20 μg/l
13.	Standard 3	50 μg/l
14.	Standard 4	100 μg/l
15.	Recalibration rate	30
16.	Calibration algorithm	New rational
17.	Total volume	15 μl
18.	Sample volume	10 μl
19.	Dilution coefficient	2

GF-AAS, graphite furnace atomic absorption spectrometry.

Samples and reagents. Biological samples (20 urine samples and 20 blood samples) were collected during hospitalization (23 days) from a patient admitted to the Intensive Care II Unit, Toxicology Department within the Bucharest Emergency Clinical Hospital. Written informed consent was obtained after the study protocol was previously discussed and explained to the patient.

All chemicals were of analytical or certified-reagent grades. Lead standard solution Certipur® (Merck) (1,000 mg/l Pb) was used. A lead stock solution (100 μg/l) was prepared daily in 0.01% nitric acid. Concentrated nitric acid (Lach-Ner), with a lead content (<0.00005%) below the GF-AAS detection limit, was used. Solutions were prepared with grade doubly distilled, de-ionized water in polypropylene calibrated flasks. The required volumes were measured with air displacement pipettes (Eppendorf Research plus Models) with premium grade polypropylene tips. All glassware was cleaned with acid and rinsed thoroughly with distilled or unionized water before use.

Blood samples. Sodium heparin (Lilly) with a lead content below the GF-AAS detection limit was used. For analysis, 200 μl of blood was mixed together with 800 μl 5% anti-foam B (Sigma-Aldrich; Merck KGaA) solution and 1,000 μl 1.6 M solution of 65% HNO_3. They were allowed to stabilize for 10 min and then centrifuged for 5 min at 2,884 x g relative centrifugal force (RCF). The supernatant was collected and

Table II. Furnace GTA-100 Varian operating conditions for lead measurements.

Step	Temperature (˚C)	Time (sec)	Flow (liters/min)	Gas type	Read	Signal storage
	40	1.0	3.0	Normal	No	No
	85	5.0	3.0	Normal	No	No
	95	40.0	3.0	Normal	No	No
	120	10.0	3.0	Normal	No	No
	120	5.0	3.0	Normal	No	No
	400	5.0	3.0	Alternate	No	No
	400	1.0.	3.0	Alternate	No	No
	400	2.0	0.0	Alternate	No	Yes
	2,100	1.0	0.0	Alternate	Yes	Yes
	2,100	2.0	0.0	Alternate	Yes	Yes
	2,100	3.0	3.0	Normal	No	Yes
	40	2.0	3.0	Normal	No	No
	40	5.0	0.0	Normal	No	No

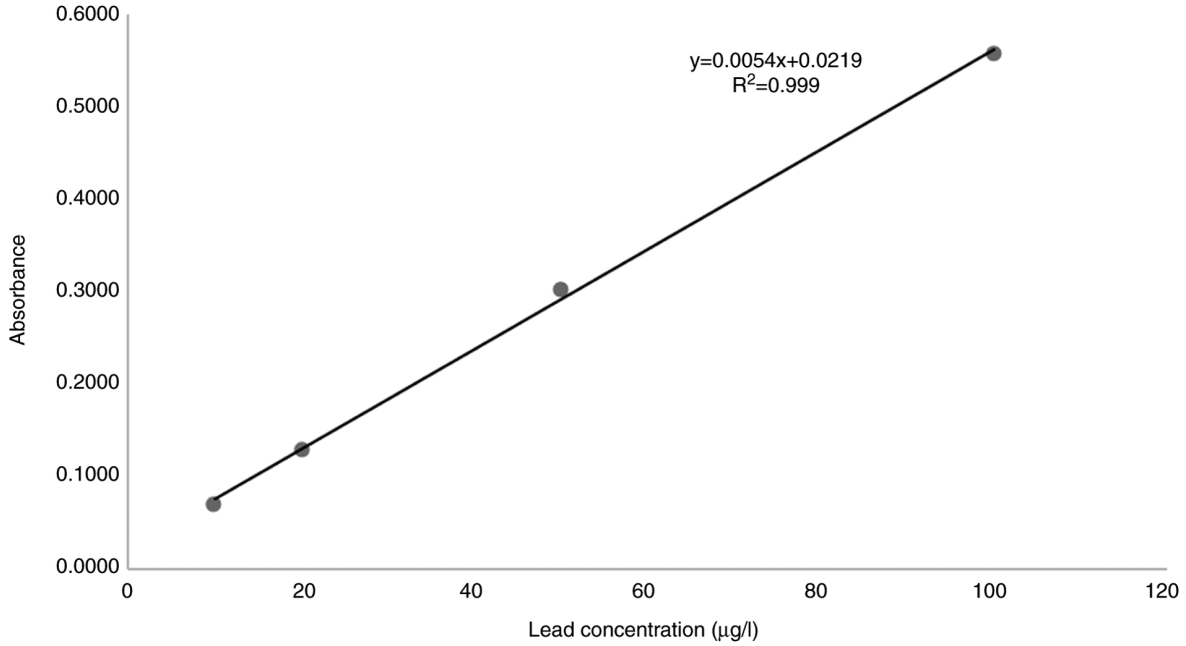

Figure 1. The linear regression curve for the determination of lead etalon model.

analyzed on the graphite furnace atomic absorption (GF-AAS) system.

Urine samples. A volume of 9 ml of urine was treated with 1 ml of 65% HNO_3. The sample was allowed to stabilize for 20 min and then centrifuged for 10 min at 2,884 x g relative centrifugal force (RCF). The supernatant was then analyzed on the GF-AAS system. There were several trials using different working parameters, to reach optimum conditions (Table I).

Results

Optimization of the working parameters. The atomization temperature was established by varying the atomization temperature between 1,600 and 2,100˚C. As expected, the lead signal increased with the increase in the atomization temperature up to 2,000˚C. For temperatures >2,100˚C, the signals remained almost constant, indicating that maximum atomization efficiency can be achieved in this range (Table II).

The application of the optimized temperature program made possible elimination of the whole matrix of the sample before the atomization step, as confirmed by the low background signals observed in the measurement of lead (13-15). To determine the performance parameters for the method (linearity, accuracy, precision and robustness) standard calibration solutions were used in the concentration range 10-100 µg/l (14). The detection and quantification limits were established according to ICH (International Conference on Harmonization) recommendations (16,17).

Table III. Performance parameters of the linear regression equation.

Denomination	Conc. (μg/l)	Abs	Media Abs	Abs without blank	R^2 coefficient of determination	Slope	Standard deviation of slope (STD)	Ordinate at origin b	Standard deviation (STD) of the ordinate at origin
Blank	0	0.0086	0.0085		0.9986	0.0054	0.0001	0.0219	0.0068
		0.0091							
		0.0079							
Standard 1	10	0.0726	0.0788	0.0703					
		0.0839							
		0.0799							
Standard 2	20	0.1393	0.1387	0.1302					
		0.1363							
		0.1406							
Standard 3	50	0.2943	0.3095	0.3009					
		0.3151							
		0.3190							
Standard 4	100	0.5744	0.5654	0.5569					
		0.5630							
		0.5589							

Abs, absorbance; conc., concentration.

Table IV. Validation parameters.

Cation	Pb^{2+}
Linearity range	10-100 μg/l
Regression equation	y=0.0054x + 0.0219
Correlation coefficient (R^2)	0.9990
Intercept	0.0219
Slope	0.0054 μg/l
SE of intercept	0.0068
SD of intercept	0.02633572
LOD	4.15 μg/l
LOQ	12.59 μg/l

SE, standard error; SD, standard deviation; LOD, limit of detection; LOQ, limit of quantification.

A linear relationship was found between the absorbance at 283.3 nm and the concentration of lead in the range of 10.0 to 100 μg/l. The representative linear equation was y = 0.0054x + 0.0219 where: y is the absorbance, x is the lead concentration (μg/l), calculated by the least squares method. The regression coefficient (R^2) standard curve was 0.9990 (Fig. 1) indicating good linearity.

The performance parameters of the linear regression equation are presented in Table III. The parameters of the GF-AAS analysis method of lead are presented in Table IV, and the calibration curve can be observed in Fig. 1. The limit of detection (LOD) and the limit of quantification (LOQ) were detected for the method based on the standard deviation of 6 readings of the standard solution blank and on the slope of the analytical curve (Table III).

LOD is the lowest concentration of an analyte that can be detected while LOQ is defined as the lowest concentration of an analyte that can be determined at the acceptable level of precision and accuracy and were calculated according to the formula below: LOD = 3.3x (SD of intercept/slope) and LOQ = 10x (SD of intercept/slope) (Table IV).

The accuracy of the assays (BIAS), expressed as the consistency between the real value and the analytical result, was calculated using three sources of blank matrix samples fortified at each level of concentration analyzed in duplicate. The calculated coefficient of variance (CV%) which describes the precision of the analytical method is shown in Table V. The CV% did not exceed 15% at each concentration and the BIAS was <15% at each level of concentration, which ensured a superior trust grade for each determination using this method. In other words, the difference between the real value and the determined value of concentration was minimal.

Clinical application. Lead level monitoring in humans is of great importance due to its high toxicity. In order to detect lead in whole blood, the authors developed a method using GF-AAS, for quantifying the lead level in blood which can be used for monitoring lead levels during chelation treatment time. The permissible concentration of lead in the blood is up to 20 μg/dl, and in urine ≤40 μg/l.

Acute lead poisoning manifests with acute abdominal pain, nausea and vomiting, being a neglected cause in the differential diagnosis of acute surgical abdomen. A careful anamnesis,

Table V. The performance parameters of the analytical method for lead determination.

Level	Standard solution concentration μg/ml	Measured concentration μg/ml	Average measured concentration μg/ml	Standard deviation	BIAS	CV%
1	10	9.41	10.56	1.06	-5.90	10.04
	10	11.50			15.00	
	10	10.76			7.60	
2	20	21.78	21.67	0.41	8.90	1.89
	20	21.22			6.10	
	20	22.02			10.10	
3	50	50.53	53.34	2.46	1.06	4.61
	50	54.38			8.76	
	50	55.11			10.22	
4	100	102.47	100.81	1.49	2.47	1.48
	100	100.36			0.36	
	100	99.60			-0.40	

BIAS, accuracy of the assays; CV%, coefficient of variance.

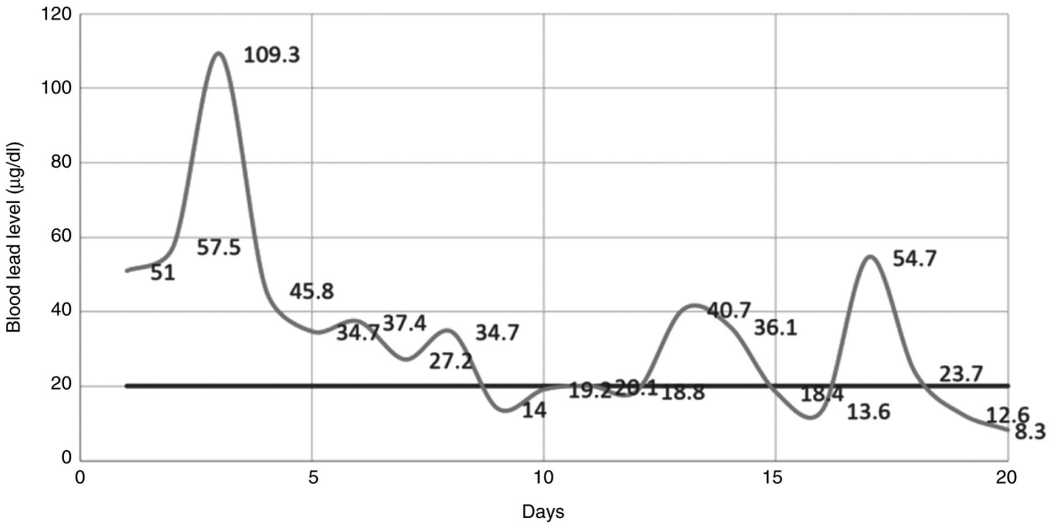

Figure 2. Variation of lead levels in whole blood, measured by the presented GF-AAS technique, during chelating treatment in a case of lead intoxication. GF-AAS, graphite furnace atomic absorption spectrometry.

revealing previous toxicological history, occupational risk or hobbies that might be associated with lead exposure, may reveal important information, but it may not be always accurate, due to patient multiple comorbidities, ignorance of the possible toxic risk or neuropsychiatric disorders. A frequent associated condition is anemia, due to the increased fragilization of the erythrocyte membranes and hemolysis. In suspect cases or patients with a non-conclusive clinical and imagistic exam, a prompt and reliable determination of lead concentration in blood is extremely important to avoid 'white' laparotomies and associated perioperative morbidity.

The therapeutic approach is based on sustaining vital functions and increased lead elimination through urine. Chelating therapy is usually initiated when lead concentration in blood is more than 50 μg/dl, due to the possible dangerous side effects.

The most commonly used chelating agents in lead intoxication include: Dimercaptosuccinic acid, dimercaprol and $CaNa_2$ EDTA. Among the current drawbacks of the chelation therapy, the clinician must take into account the hepatotoxicity and nephrotoxicity, essential metal loss, headaches, nausea, arrythmias, hypotension or hypertension, bone marrow depression, convulsions or even cardio-respiratory arrest (17,18). Another aspect is the redistribution of the lead among the body compartments, which may generate important fluctuation of blood and urine concentration during the first days of therapy. Prolonged treatment with $CaNa_2$ EDTA results in depletion of essential metals, especially Zn, Cu and Mn, requiring oral Zn supplementation (17).

Chelating therapy requires close monitorization and advanced medical skill (5). In our department, patients admitted for acute lead poisoning are closely followed

up by daily laboratory tests including: Hemoleucogram, transaminase, urea, creatinine, electrolytes (Na^+, K^+, Ca^{++}), as well as lead concentration in the blood and urine. The optimized method based on GF-AAS technique proves to be useful for monitoring chelation therapy in the cases of acute lead poisoning. It allows a close follow-up of the dynamic concentrations of lead in the blood and urine, as presented in Fig. 2.

Discussion

Saturnine colic is an infrequent cause of differential diagnosis of acute abdomen, which may appear in cases of acute lead poisoning, with blood lead levels exceeding 50-80 μg/dl (2,19-22). If undiagnosed, it can lead to avoidable surgeries, by mimicking acute appendicitis, perforated ulcer, acute pancreatitis or bowel obstruction (21-23). The abdominal pain is intense, colliquative, in the periumbilical area, resistant to usual antispastics (2,20-22). The pain diminishes at profound palpation of the abdomen, with no tenderness or contracture, being a key element of differential diagnosis with surgical acute abdomen. Other signs and symptoms are anorexia, nausea, vomiting, constipation or very rarely, diarrhea (23,24). Radiological abdominal exam shows hydroaeric images, with alternative sectors of spastic and moderate dilated intestinal loops. The patient may experience transient increased blood pressure, which comes back to normal after ceasement of abdominal pain with chelating therapy. Oliguria, increased serum urea and leukocytosis may be associated with acute poisoning (19).

It is well known that exposure to lead causes dose-dependent decreases in heme synthesis by inhibiting the enzyme δ-aminolaevulinic acid dehydrase (δ-ALAD). Hematologic tests such as hemoglobin concentration may suggest toxicity, but this is not specific for lead (25). The higher the level of blood lead levels, the lower the hemoglobin in the blood and the level of erythrocytes due to the increase in lead-induced membrane fragility resulting in the development of anemia (26). The toxicity of lead on the human hematological system has been established in numerous studies. Other effects of lead on the hematological system are decreased activity of erythrocyte enzymes (pyrimidine 5'-nucleotidase) and altered levels of plasma erythropoietin (27).

The effects of acute and chronic lead intoxication upon the nervous system have been studied for 100 years. Lead is a highly neurotoxic element, both for central nervous system and peripheric nerves. Even concentrations below 10 μg/dl, in children, are inversely correlated with the intelligence quotient (IQ). There are well-defined clinical features encountered in both adults and children: Decreased learning ability, memory loss, cognitive deterioration, reduced neural signaling and demyelination. At blood levels >70 μg/dl in children and >100 μg/dl in adults lead toxicity is increased and may cause paresis or paralysis and saturnine encephalopathy, with sudden seizures, changes in consciousness, coma and death (28,29). Furthermore, several studies have confirmed the pathogenic role of lead intoxication in Alzheimer disease and glaucoma, by increasing the tissular oxidative stress, through depletion of glutathione and thiol pools, as well as by disrupting the antioxidant defense system (30-35). Several studies revealed that probiotics may be useful for alleviation and treatment of lead toxicity, reducing the specific side-effects in heavily polluted areas (36,37). Accidental or occupational lead intoxication is an important public health problem, causing a significant burden especially in low- and middle-income countries (38). The Institute for Health Metrics and Evaluation (IHME) estimated that in 2017, lead exposure accounted for 1.06 million deaths and 24.4 million years of healthy life lost worldwide due to long-term effects on health (39). The most severe include the neurologic and cardiovascular effects of acute or chronic lead poisoning: 63.2% of the global burden of idiopathic developmental intellectual disability, 10.3% of the global burden of hypertensive heart disease, 5.6% of the global burden of the ischemic heart disease and 6.2% of the global burden of stroke (40).

Graphite furnace atomic absorption spectrometry (GF-AAS) is increasingly becoming the method of choice for the determination of lead and other heavy metals in blood and urine, as well as in other biological products (40-43), with several improvements developed in time to increase its power of detection and determination for lower concentrations.

Whole blood lead levels are the most widely used and most generally accepted measure of absorbed dose. A repeatable, reliable, cost-efficient method is an important tool for lead intoxication screening and chelation therapy monitoring in clinical practice.

GF-AAS is simpler, less expensive, quicker and more accurate than neutron activation or emission spectrometric technique. The absorbance signals obtained for lead in the optimizing conditions presented ensures a well-defined profile and a low background (42-44).

The reported method shows high precision and accuracy, as well as a wide applicability in routine lead determination and research assays. The methods developed are valuable for clinical diagnostics and biological monitoring of work-related exposure.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MST, GC, OA, ADS and DS were responsible for the conception and design of this study. DMP, CGS, CT, AMD, CDB, DOC and BS were responsible for the data collection and analysis. MST, GC, OA, CT and DS were in charge of drafting the manuscript. AMD, MC, ADS, DOC and RS revised critical perspectives for important intellectual content. The final version for publication was read and approved by all the authors.

Ethics approval and consent to participate

Written informed consent was obtained after the study protocol was previously discussed and explained to the patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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