An Overview of the Laboratory Diagnosis of Lead Poisoning

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ABSTRACT

Four tests for the evaluation of lead poisoning are reviewed from both the clinical and methodological aspects. Whole blood or erythrocyte lead measurements appear to provide the best means of assessing the bodily burden of lead with electrothermal and Delves cup flame atomic absorption spectrophotometric techniques providing accurate and precise results. Urine lead is less reliable as a screening test for lead poisoning but is excellent for monitoring the course of ethylenediamine tetraacetic acid (EDTA) therapy.

Atomic absorption methods for urine are made difficult by the variable matrix of urine but satisfactory electrothermal and flame procedures have been described. Erythrocyte δ-aminolevulinic acid dehydratase activity is a very sensitive index of lead exposure,—perhaps too sensitive. Analytical procedures for measuring this enzyme are subject to errors and many complicating factors such as lack of stability of the specimen limit the usefulness of the test. Urine δ-aminolevulinic acid is of questionable value as a screening procedure and also is subject to analytical problems.

Clinical Value of Tests for Monitoring Lead Exposure

Lead poisoning is an extensive problem affecting selected segments of the population. The greatest attention has been paid to children living in areas of low income housing in urban areas where poisoning results from ingestion of lead based paints. Industrial lead poisoning in workers employed in some manufacturing processes has been recognized, and the consumption of “moonshine” contaminated with lead has resulted in many cases of lead poisoning.

This present report reviews the following four tests which have been widely used for monitoring exposure to lead: (1) whole blood or erythrocyte lead, (2) urine lead, (3) erythrocyte δ-aminolevulinic acid dehydratase (δ-ALAD) and (4) urine δ-aminolevulinic acid (δ-ALA). All of these tests have strengths and limitations depending on the situation to which they are applied such as clinical or industrial applications.

WHOLE BLOOD OR ERYTHROCYTE LEAD

There is fairly wide agreement that whole blood lead levels are an accurate index of lead exposure under known conditions of route and duration of exposure, and type and amount of the lead compound absorbed. Beyond these well
defined situations, there is much uncertainty over the value of blood lead determinations in cases of suspected exposures. Several studies have used 80 μg per dl as a cutoff level in the clinical assessment of lead toxicity and numerous false negatives have been observed.\textsuperscript{48,50} Kehoe\textsuperscript{26} proposed a threshold concept for lead exposure in which levels of 80 μg per dl and less were tolerated by man while levels above this would lead to toxic effects. Waldron\textsuperscript{52} agreed with the threshold hypothesis but believed that the level should be lowered to 50 μg per dl for adults and 30 μg per dl for children. Several studies would have reached very different conclusions regarding the extent of lead poisoning had such limits been used.\textsuperscript{48,50}

In a study on baboons,\textsuperscript{18} various doses of different lead compounds were administered over a 150 day period and both blood lead and δ-ALAD levels were measured. There was a very definite, though gradual, rise in blood lead concentrations with duration of lead exposure. This was contrasted with rather sudden drops in δ-ALAD activity observed shortly after onset of lead administration which remained essentially constant at low activities throughout the remainder of the experiments. Many studies\textsuperscript{30,39,42,44} have shown moderate correlations between blood lead levels of up to 60 or 80 μg per dl and δ-ALAD activities. It would appear from these studies that blood lead levels are very good indicators of lead exposure and/or absorption over a wide range of lead exposures. This is contrasted with δ-ALAD which differentiates rather poorly among very large lead absorptions.

An unanswered question is whether whole blood or erythrocyte lead levels are a better index of lead intoxication. It is well known that 95 percent or more of lead in blood is bound to erythrocytes.\textsuperscript{8,23} It is also known that inhaled lead\textsuperscript{41} and in vitro absorption of lead\textsuperscript{24} will rapidly reach equilibrium in the red cell. A recent paper\textsuperscript{27} has shown that erythrocytes have a large capacity for lead. In fact, for hematocrits studied between 20 and 65 percent, the hematocrit was not a determinant in the amount of lead absorbed. That is, the red cell has a very large capacity for binding lead and even for a hematocrit of 20 percent the red cell binding sites appear to be in great excess up to extremely high lead concentrations.

Pertinent to this situation was a case noted by Farellly and Pybus\textsuperscript{15} in which a worker with severe lead poisoning had a whole blood lead of 75 μg per dl but a red cell lead of 370 μg per dl. Evenson and Pendergast\textsuperscript{14} also have measured erythrocyte lead levels on normal subjects and obtained a range of 12 to 70 μg per dl. In the authors' laboratory, a similar normal range for erythrocyte lead has been observed and several patients have been found to have erythrocyte lead levels of approximately 100 μg per dl without demonstrating abdominal pain or neurological symptoms.

Thus, blood lead measurements constitute an excellent method for screening all types of persons with unknown exposures to lead. There does not appear to be any practical saturation level of lead, which continues to rise with increasing exposure. For several reasons described, the analysis of packed erythrocytes appears to have advantages over whole blood analysis, and it is regrettable more studies have not included this measurement when studying lead exposures.

The current most widely used methods for lead measurements involve atomic absorption techniques and an evaluation of several procedures has been described by Anderson and coworkers.\textsuperscript{2} Some of the earlier procedures to be described used flame atomization and required either protein precipitation or digestion followed by chelation and extraction of lead.
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into an organic solvent. Sample requirements ranged from 0.25 ml to 20 ml. Other methods eliminated any precipitation or digestion steps prior to chelation-extraction and required from 2 to 6 ml of sample. The "Delves cup" atomic absorption procedure and its modifications employed microliter amounts of blood and required either a digestion or charring step on the cup. These methods provided quite specific techniques for lead analysis although in most cases blood standards were required to provide optimal accuracy.

More recently, the graphite tube or rod electrothermal atomization devices have provided the potential for direct determination of lead in blood with excellent sensitivity and also have permitted the use of aqueous standards. Evenson and Pendergast have studied the matrix interferences which they eliminated to a large extent when measuring erythrocyte lead. However, using the same techniques on whole blood they observed less than quantitative recoveries of lead. Kubosik et al also employed electrothermal atomic absorption techniques and initially reported recoveries of 96 percent for whole blood lead using aqueous standardization. Later they found that aqueous and blood lead standards did not give the same analytical curves, and standards made up in a blood matrix were necessary. One great advantage of electrothermal atomization techniques is the small sample requirement, which permits analysis on blood obtained by capillary puncture. It appears that sample requirements are limited principally by the ability of the investigator to handle these micro samples. Cemik reported a method for collecting blood from a finger puncture on a piece of filter paper, a disc of the dried blood being punched out and analyzed directly. Samples collected in this manner could be mailed to a central laboratory facility for analysis. The principle error in electrothermal atomization techniques appears to result from imprecision in sample handling, particularly in pipeting microliter volumes of blood onto the graphite rod. With practice, however, precision on the order of 5 percent (RSD) is attainable. Atomic absorption, therefore, appears to be the method of choice for determination of lead in blood and many advantages are to be found in electrothermal atomization and the Delves cup flame atomization techniques.

Urine Lead

Urine lead determinations appear to be of limited usefulness as a screening procedure for lead poisoning. In addition to presenting an unusually difficult matrix for analysis, urine lead may not reflect the degree of lead absorbed or lead toxicity. The usefulness of lead determinations is subject to conflicting reports. It appears that urine lead measurements give very few false positives but might miss several patients with moderate or high lead exposures. One study found 24 of 25 urine lead values to be normal in patients later shown to have increased body burdens of lead demonstrated by large excretions of lead following administration of EDTA. This investigation also demonstrated the considerable value of urine lead measurements during EDTA therapy, confirming the effectiveness and course of therapy.

In another report, two moonshine drinkers with symptoms of severe lead poisoning had non-toxic blood lead levels yet were excreting elevated amounts of lead in the urine, thus indicating that the urine measurements were sensitive indicators of lead toxicity in these two instances. However, in a study of lead antiknock workers, the conclusion was that urinary lead was a more sensitive indicator of organic lead exposure than was urinary δ-ALAD. Lauwerys et al found a
correlation between urine lead and blood lead levels in occupationally non-exposed subjects. Selander and Cramer also found these measurements to be correlated in occupationally exposed workers. Kehoe states the actual earliest sign of the absorption of lead is a rise of urinary lead followed somewhat later by blood lead.

Thus, the determination of urinary lead, with some reservations, may be of value in monitoring lead workers, especially those working with organic lead. Urine lead levels within an individual are subject to wide variation, especially on spot collections which is a definite drawback. However, the ease of collection of spot urine specimens is one obvious advantage when considering a screening method. The monitoring of urine lead output throughout chelation therapy is very helpful as a guide to therapy and is also a definite diagnostic method to confirm an increased body burden of lead.

It has been the authors' experience that urine presents an unusually troublesome matrix for measuring lead by electrothermal atomic absorption spectrometry. Addition of perchloric or nitric acid improves sensitivity, although recoveries relative to aqueous standards are highly variable. Kubasik and Volosin reported a chelation-extraction method for measuring urinary lead, cadmium, and thallium, but low recoveries were obtained with aqueous standards and, therefore, the method of standard additions was used. Various flame atomic absorption methods have been described which use chelation-extraction procedures to quantitate the lead in urine samples. Aqueous standards were used in these methods which apparently provided accurate results although the complexities of these methods with extensive numbers of sample manipulations were definite drawbacks. Anderson and Mesman reported a direct method for urine lead which compared the signal from a urine sample with the signal obtained from a pooled urine sample with a known lead content. Very small amounts of urine were required and analysis was relatively rapid.

**ERYTHROCYTE δ-AMINOLEVULINIC ACID DEHYDRATASE**

There appears to be almost universal agreement that δ-ALAD is a very sensitive, early and quite specific indicator of lead absorption. In most cases there has been a good correlation between increases in blood lead levels and decreases in δ-ALAD activity. However, this relationship appears to hold only for relatively low concentrations of lead in blood (≤ 80 μg per dl or below). It appears that the δ-ALAD activity drops quickly upon exposure to lead. As the degree of lead exposure increases even up to dangerous levels, the δ-ALAD rate of decrease seems to level off and reach a stable activity which is near the detection limit for the method. As a further hindrance to interpretation, δ-ALAD has been shown to exhibit different pH optima depending on whether the source is a "nonexposed" individual or one who has had an occupational or some other distinct lead exposure.

Tomokuni found that if the reaction were carried out at pH 6.0 (the optimum pH for the lead exposure δ-ALAD), the δ-ALAD activity was actually greater than that found in normals. When he applied an activity ratio, little improvement was obtained. Tomokuni later observed that heat treatment would shift the pH optimum for δ-ALAD from lead workers up to about pH 6.6 and increase the activity about 3.6 fold. Granick et al found inhibition by lead was fully eliminated by treatment with dithiothreitol, and the decrease in activity of δ-ALAD was due not to any decrease in enzyme levels but rather to a noncompetitive inhibition by
lead. The activity of δ-ALAD also has been shown to be unstable under certain conditions of storage. Prpic-Majic et al found samples should be analyzed within two hours after collection or else stored at 1° and a correction made for the loss in activity expected with time. Granick et al found δ-ALAD to be stable up to 48 hours when stored at 4° and large losses were encountered with storage at either +25° or −20°. In all cases the activity could be restored by dithiothreitol.

Thus, the use of δ-ALAD appears to be potentially the most sensitive available means for detecting lead exposures. This very sensitivity seems to hinder its wider use, in that marked changes in δ-ALAD can occur with very low, apparently harmless, exposure to lead. In cases of severe lead absorption the δ-ALAD appears to be too insensitive since its activity tends to level off as the amount of absorbed lead becomes high.

Several methods have been devised for assaying δ-ALAD activity in erythrocytes based upon the conversion by δ-ALAD of two molecules of δ-ALA to porphobilinogen which has been measured spectrophotometrically using Ehrlich’s reagent. Burch and Siegel modified the Bonsignore procedure to eliminate the pH shift occurring during the incubation which led to losses of δ-ALAD activity. Tomokuni modified these procedures slightly and measured the decrease in δ-ALA, rather than the increase in porphobilinogen, in order to correct for porphobilinogen breakdown to porphyrins occurring during the analysis. In this method the δ-ALA-pyrole was extracted with Ehrlich’s reagent, the extraction being necessary to remove porphobilinogen which interfered in the color reaction.

These methods for δ-ALAD are rather time consuming, require blank corrections and the use of Ehrlich’s reagent for colorimetric development is susceptible to many interferences. In addition, the ready inactivation of the enzyme by sulfhydryl oxidation makes proper storage and rapid analysis imperative. The different pH optima observed in δ-ALAD from lead exposed and nonexposed individuals also introduces another variable in the analysis. Thus, the problems inherent in the measurement of δ-ALAD constitute a major obstacle in using this test for detecting lead poisoning.

Urine δ-Aminolevulinic Acid

Urinary δ-ALA excretion has been used extensively as an indicator for lead exposures near or above potentially harmful levels where it has been noted that elevations of δ-ALA occur. However, conflicting reports have appeared in the literature. For example, Tomokuni in a study of ninety-one workers occupationally exposed to lead found a poor correlation between urinary δ-ALA levels and either erythrocyte δ-ALAD or blood lead. These workers, however, were exposed to relatively low levels of lead, their mean lead concentration being 18.4 μg per dl with a mean urinary δ-ALA of 2.57 mg per l. Vitale et al studied thirty workers engaged in lead refining or welding and in general found that urinary δ-ALA was rather insensitive to lead absorption as measured by urinary lead excretion after EDTA treatment.

A Public Health Service report concluded that urinary δ-ALA was subject to wide analytical variation and scatter of normal ranges. In contrast, Kehoe indicated that δ-ALA was a useful indicator of lead absorption and could be used for monitoring lead workers. Indeed, Davis et al in a study of 250 children with suspected lead absorption found that δ-ALA correlated well with a diagnosis of normal lead absorption. The 250 subjects studied by these workers were selected based upon the result of a blood lead
screening test of above 40 μg per dl. It was noted that normal δ-ALA levels were associated with blood lead levels of less than 60 μg per dl and abnormal δ-ALA levels corresponded to lead levels generally above 60 μg per dl. Finally, Robinson concluded the δ-ALA values in urine were of uncertain value in monitoring workers exposed to organic lead compounds and urinary lead measurements were more sensitive indicators of exposure to organic lead.

Several methods for the measurement of δ-ALA in urine are available, most of which are based upon the condensation of δ-ALA with acetylacetone to form a pyrrole which reacts with Ehrlich's reagent to form a pink colored complex. The Mauzerall-Granick procedure used an ion-exchange column to isolate δ-ALA for analysis but was extremely time consuming. The Davis-Andelman modification used disposable ion-exchange columns and was capable of up to 40 analyses per hour. Grabecki et al proposed a method without use of an ion-exchange column and eliminated interferences by analysis of an appropriate blank, but other workers have criticized the precision and specificity of this method.

Lauwerys et al have shown that substances present in urine interfere differently from one urine to another with the Ehrlich color development. An automated method was described by them for δ-ALA without use of ion-exchange chromatography. In their method a known amount of δ-ALA was added to each sample before analysis and, reportedly this method was less susceptible to interferences. Tomokuni and Ogata used an extraction step with ethyl acetate to separate the ALA-pyrrole produced by the condensation reaction with other Ehrlich positive substances remaining in the aqueous phase. Berlin et al evaluated δ-ALA results on urine samples distributed to 26 European laboratories.

None of the methods used were precise or accurate enough to detect the small differences in δ-ALA excretion which one would expect to see in patients exposed to environmental lead. This study showed a considerable scatter in results which was attributed to difficulties in analytical technique rather than to intrinsic problems with the methods themselves. Roels et al compared four δ-ALA methods and although correlation coefficients were generally high between all methods, there was significant proportional and/or constant error between all methods. They found certain buffers (TRIS) could give widely different results between some methods. These workers recommended storage of samples in the dark at 4°, and under these conditions urine δ-ALA was stable for two weeks.

Summary

It is apparent that all four methods described for the assessment of lead exposure possess merits and faults when factors such as accuracy, precision, specificity, time of analysis and clinical usefulness are considered. The determination of lead in blood (erythrocytes) by atomic absorption using the Delves cup or electrothermal atomization techniques is highly specific and has good accuracy and precision although technical difficulties may be encountered. Contamination by extrinsic lead is a problem at every step of the collection and analysis. An accurate value for lead in the blood nevertheless is very helpful to the clinician in making a diagnosis and in following patients exposed to toxic levels of lead.

The measurement of urine lead by atomic absorption techniques seems to have a requirement for standards prepared in a urine matrix. This highly variable matrix of urine almost certainly affects the accuracy of the methods. The
use of urinary lead as a screening procedure has given inconsistent results. Although the collection of urine is relatively simple, the wide variation in excretion of lead hinders its reliability. Urine lead determination after and during chelation therapy is perhaps the best method for confirming increased body burdens of lead and for following the course of therapy.

The activity of δ-ALAD in erythrocyte is a very sensitive indicator of lead absorption. This observation, along with lack of contamination problems, makes it a potentially useful test for screening purposes. However, it is possible that δ-ALAD activity is too sensitive to lead. One should be aware of the stability problems and variation owing to pH optima which exist. Lastly, the specificity of the methods using Ehrlich’s reagent is questionable.

The use of Ehrlich’s reagent in the measurement of δ-ALA in urine presents similar problems with specificity. The methods for δ-ALA usually employ some means of correcting for or eliminating the interferences present in urine. Such manipulations appear to affect adversely the precision and accuracy of the methods and this variation has presented problems in its use. Overall, δ-ALA is of questionable value as a screening procedure.

References


