THE LEAD LABORATORY

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Role of the Laboratory

Laboratories measure lead concentrations in either clinical samples such as blood and urine or in environmental samples such as paint and dust. It is important to distinguish between the clinical and environmental lead laboratories and the issues that they face. Often, laboratories are separated according to the types of samples they analyze even though the technologies used to measure lead levels may be similar.

Several reasons exist for separating laboratories along sample lines. For example, clinical and environmental laboratories operate under different federal or state regulations. Sample handling and reporting requirements of state and federal agencies differ, as do requirements for quality control. The reportable concentration range for lead in paint or in dust, and the preparation of the sample requires different procedures.

Further, the higher concentrations of lead typically found in lead-based paint (>0.5% by weight) and in lead contaminated dust (>1 mg/g) present the potential for contaminating the laboratory environment. If environmental and clinical laboratory functions are carried out in the same laboratory room, determination of the much lower concentrations of lead found in blood is jeopardized.

For all *clinical* laboratories in the United States, the Health Care Financing Agency (HCFA), operating under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), regulates all aspects of laboratory operation including determining the qualifications of a laboratory director and establishing protocols for quality assurance and quality control (QA/QC) activities, method validation, specimen collection, storage, analysis and reporting of results (42 CFR Part 493). For blood lead laboratories specifically, successful participation in an approved proficiency testing (PT) program is required. In addition, many states regulate the operation of clinical laboratories under their jurisdiction although the requirements vary greatly from state to state. Laboratories

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are advised to contact their state health department to ensure compliance with state and local laws.

For *environmental* laboratories, the primary regulatory agencies are the U.S. Environmental Protection Agency (EPA) and the National Institute for Occupational Safety and Health (NIOSH). In many laboratories, the EPA's Contract Laboratory Program (CLP) requirements drive all QA/QC activities and restrict the analytical protocol used. Several states also regulate environmental laboratories under their jurisdiction, although regulations vary from state to state.

The clinical lead laboratory

Determining lead levels in blood
A blood lead test is recommended for initial screening purposes, and for diagnostic evaluation. Serial blood lead measurements are recommended for monitoring patients under medical management.

Specimen collection
Capillary blood lead measurements may be used for initial screening purposes, but only venous blood is appropriate for diagnostic evaluation, and a venous BLL is necessary before initiating an environmental investigation or chelation therapy. From 1991 to 1994, CDC sponsored several studies to evaluate the performance of capillary blood lead screening by comparing blood lead levels in specimens obtained by fingerstick with those obtained simultaneously by venipuncture.

Results of studies conducted by Yale New Haven Hospital and the Connecticut Department of Health, the City of Milwaukee, and the New York State Department of Health, independently concluded that, *provided that a strict cleaning protocol is followed*, capillary whole blood obtained by fingerstick is a reliable specimen for pediatric lead screening purposes.

Most capillary blood specimens that show falsely elevated lead levels can be traced to inappropriate collection procedures or to contaminated materials used to collect and transport the specimen[1]. For these reasons, laboratories should ensure that all materials used to collect capillary blood specimens are free from significant lead contamination. It is recommended that capillary blood be collected in plastic microcollection devices containing either EDTA (lavender caps) in powder form or heparin (green caps) rather than glass capillary tubes, which can break and may result in injury and disease transmission.

The choice of microcollection container is a matter for each individual laboratory and depends on factors such as the desired sample volume or anticoagulant type. Although capillary blood is useful for initial screening, only venous blood should be used for diagnostic evaluation or medical management. A recommended procedure for collecting capillary blood by fingerstick is given in Appendix C.2.

Recommended procedures for controlling contamination

Perhaps the most frequent source of error is contamination by airborne particulates containing lead. Although use of lead in gasoline and paint has been reduced, lead still remains a ubiquitous pollutant, especially in common dust. Therefore, special precautions must be taken to prevent contamination during specimen collection and analysis. These precautions include checking all collection materials and supplies for significant contamination and using dust-control measures in the laboratory as recommended below.

Some manufacturers provide blood collection tubes specifically for trace element analysis (royal blue caps) or certified lead-free tubes for blood lead level determination (tan/brown caps). Laboratories should check lead-free claims made by individual manufacturers before using such devices since the amount of lead in these devices is reported to vary greatly.

Once a reliable source of certified lead-free materials has been located, laboratories need not check each individual lot for lead contamination. Since the cost of certified lead-free tubes is likely to be much more than for standard tubes, some laboratories may prefer to obtain the standard tubes and certify them as lead-free for their clients as described below. This strategy will hold down the costs associated with blood lead level (BLL) screening.

Checking collection materials and supplies

Two approaches may be used to assess contamination and ensure that materials are not significantly contaminated with lead. First select at least 10 collection devices at random from a batch to test. Then take either of these two steps:

- Fill specimen or sample containers with either dilute acetic acid (4% v/v) or dilute nitric acid (2% v/v) and store for 24 hours at room temperature. Analyze leachate for lead and calculate the total amount of lead extracted.

- Fill specimen or sample container with a base low-lead blood sample of known lead concentration, and store for a period and under conditions the the laboratory maintains for routine patient samples. Analyze the blood lead levels to ascertain whether or not any significant lead contamination has occurred.

For both methods, any measurable increase in lead concentration should amount to no more than 0.5 µg/dL (i.e., 5% at 10 µg/dL). Needles, lancets, and materials other than containers should

also be checked for gross contamination by leaching with a minimum volume of dilute acetic or dilute nitric acid and analyzing the leachate for lead. Generally, such materials should be free of significant contamination (i.e. <1 µg/L or roughly the detection limit by graphite furnace atomic absorption spectrometry [GFAAS]).

In the laboratory, the handling of all blood is governed by the Occupational Safety and Health Administration's (OSHA's) blood borne pathogens rule (29 CFR Part 1910.1030), which specifies that CDC Universal Precautions must be used. For example, blood dilutions may be performed in Class II biosafety cabinet using powder-free protective gloves. This procedure will not only protect the analyst from pathogens but will also protect the sample from airborne lead contamination.

Most clinical laboratories are not equipped with Class 100 air or better, nor do they need to be, as long as some basic consideration is given to the potential for airborne contamination at the bench. Autosampler vials should be screened for lead contamination until a lead-free supply is verified and should be stored protected from dust (e.g., in sealed plastic bags). Autosampler vials that are in use should be protected with appropriate dust covers. Frequent wet-mopping of laboratory floors and wet-wiping of other flat surfaces will minimize contamination from airborne dust particulates.

Transporting or shipping blood specimens to centralized laboratories

Currently, most blood lead testing is performed by a centralized laboratory that is licensed for this purpose. Consideration needs to be given to the most cost-effective and reliable way to transport specimens to the laboratory. Although some laboratories may use a network of couriers to transport specimens to the laboratory, others rely on the United States Postal Service (USPS) or commercial carriers to deliver specimens. Transport of etiologic agents (e.g., human blood) via USPS is regulated (42 CFR Part 72), and many commercial carriers may have similar restrictions.

Federal regulations require that blood specimens be packaged according to guidelines requiring the use of tertiary containment and sufficient absorbent material in the event that the specimen leaks during transit. When mailing glass tubes containing blood, ensuring that the tubes cannot touch during transit may reduce chances of breakage.

Venous blood specimens preserved with EDTA or heparin are reportedly stable for determining lead levels in blood for up to 10 weeks if they are refrigerated at 4°C[2]. Refrigerated temperatures are not necessary for mailing blood lead specimens. However, where significant delays are expected, as might occur over a holiday weekend, it would be prudent to store the samples locally, refrigerated at 4°C, and then ship them the next business day. In special circumstances, such as *stat* requests, blood specimens should be mailed overnight and the laboratory forewarned to expect them.

Analytical Methods for Determining Blood Lead Levels

Several analytical methods have been applied successfully to the determination of lead in blood. The methods most commonly in use today are either GFAAS or anodic stripping voltammetry (ASV). Other analytical methods include variations on AAS, such as methylisobutylketone (MIBK)-extraction flame AAS,[3] Delves-cup microsampling flame AAS,[4] and an inductively-coupled plasma interfaced to a quadruple mass spectrometer (ICP-MS).

Beginning in 1992, CDC funded several innovative research projects aimed at developing portable instrumentation for determining lead levels in blood. Techniques under investigation include attempts to miniaturize AAS and atomic emission spectrometry (AES) and to develop various electrochemical approaches and polymeric sensors. Although each of these techniques has advantages and disadvantages, and although the new technologies may result in commercially available instruments in the future, we review only GFAAS and ASV in detail here.

Graphite Furnace Atomic Absorption Spectrometry

GFAAS has been successfully used to determine lead levels in blood.[5-8] Modern furnace instrumentation is reliable, accurate and precise and can be reasonably automated. Several manufacturers currently market GFAAS instruments that are readily configured for blood lead testing. Most of these instruments are also capable of measuring many more elements.

Selecting suitable GFAAS instruments should be done after a careful evaluation of available commercial instruments. Prospective users should request that the manufacturers provide references from customers currently performing blood lead determinations and provide evidence of successful participation in an approved PT program for measuring blood lead levels. Numerous instrumental configurations and features are available.

Most commonly employed instruments use either the Zeeman-effect or continuum background correction systems, and some have successfully used the Smith-Heifjte background correction system. Each system can correct for nonspecific background at 283.3 nm, the principal analytical wavelength for lead.

Using the Zeeman-effect background correction system is more straightforward than using a continuum source; although the latter is less expensive, it can be difficult to optimize since two radiation sources (hollow cathode and continuum) are used, both of which must be carefully aligned along the optical path of the graphite furnace.

Many GFAAS methods for measuring blood lead levels using a variety of furnace instruments[9]

have been published.* Until recently, it was thought that no one method for determining blood lead levels could be recommended for all instrumentation because of the complexity and differences between furnace equipment from the various manufacturers. However, recent experience has shown that a common or standard method for determining blood lead by GFAAS is possible.[8] This method, which uses longitudinal Zeeman background correction and a transversely-heated furnace draws upon the work of both Pruszkowska-et al.,[5] and Miller et al.[7] This simple, but rapid Zeeman AAS method for measuring blood lead levels has been successfully transferred to a simpler furnace arrangement that uses continuum background correction[10] and to other furnace AAS equipment using transverse Zeeman, continuum, and Smith-Heifjte background correction systems[11,12.]

Such standard methods are successful because they follow the stabilized temperature platform furnace (STPF) concept.[13] For a detailed description of the STPF approach to GFAAS method development, consult the appropriate references.[13,14] One critical component of the STPF approach is use of a suitable matrix modifier, which is required to stabilize lead during pyrolysis at temperatures exceeding 600°C and which will increase the volatility of the interfering matrix. Ammonium phosphate, either in the monobasic, $NH_4H_2PO_4$, or dibasic form of ammonium phosphate, $(NH_4)_2HPO_4$, along with Triton X-100 is now the most widely used modifier for determining blood lead levels by GFAAS.

Another important aspect of the STPF approach calls for using the L'vov platform for atomization coupled with integrated peak areas for absorbance measurements. Optimizing lead atomization from the platform rather than from the wall reduces gas-phase interferences and permits calibration against aqueous lead standards. Precision is also much improved over peak height measurements.

Most modern GFAAS instruments are equipped with auto samplers, which are useful for large numbers of samples and provide for unattended operation with better precision. The number of replicate firings that should occur depends on the method within-run precision, which should be less than $\pm$ 0.5 μg/dL at 10 μg/dL (5%). Replicate firings are recommended if the within-run precision is greater than 10%. When using the Delves-cup microsampling flame AAS method, where the within-run precision at 10 μg/dL can be as much as 40% ($\pm$ 4 μg/dL), carry out the analysis in triplicate. Note that this precision is different from the concept of analytical accuracy, for which federal and some state authorities require results of performance samples to be accurate to within $\pm$ 4 μg/dL (or $\pm$ 10%, whichever is greater) of the established target value.

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*A selected list of methods of measuring blood lead levels and obtaining information about instrument manufacturers and other information pertinent to the clinical laboratory is available from CDC's National Center for Environmental Health, Division of Environmental Health Laboratory Sciences, Nutritional Chemistry Branch, MS F18, 4770 Buford Highway, N.E., Atlanta, Georgia 30341-3274, telephone (770) 488-4452.

Other spectrometric techniques have been used to determine blood lead levels with varying degrees of success. Inductively coupled plasmamass spectrometry (ICP-MS) is a powerful technique that not only can detect very low concentrations of lead but can also identify and quantify the lead isotopes present. The use of ICP-MS to determine blood lead levels includes limited attempts at source identification through isotope ratio fingerprints.[15] Unfortunately, this technique is currently an expensive solution that is not cost effective for routine blood lead measurements and is limited to a handful of specialized research laboratories.

Flame AAS, with chelation of lead and its extraction into MIBK solvent, is an older technique that requires a relatively large volume (5-7 mL) of venous blood for analysis.[3] Delves-cup microsampling flame AAS was developed specifically for determining blood lead levels in small blood volumes,[4] but, since the equipment and supplies are no longer available from AAS instrument manufacturers, it has been largely replaced by GFAAS.

Anodic stripping voltammetry (ASV)

Anodic stripping voltammetry (ASV) is a manual electrochemical method capable of measuring many metals in a variety of matrices. ASV has been used to determine blood lead levels for more than 25 years. Several versions of instruments that are capable of performing this type of analysis are commercially available. The ASV technique is straightforward, and the instrumentation is relatively inexpensive, but reagent cost per test is higher than for GFAAS. ASV instruments are small, occupying less bench space than GFAAS, and require no special facilities. (GFAAS generally requires a 220 VAC, 30 A power supply.)

The analytical principle requires that lead be decomplexed and available for plating as the free +2 aqueous cation (Pb^{2+}_{aq}).
One approach is to digest (wet ash) blood specimens with nitric acid, a time-consuming and laborious preanalytical step. More commonly, blood specimens are incubated in a solution containing several other metal ions that compete with lead for typical binding sites (proteins, amino acids, EDTA, and other biological ligands), and thus displace lead as the free cation.

One ASV instrument manufacturer provides a proprietary decomplexation solution along with two controls (or, more appropriately, calibrators) specifically for blood lead analysis. To perform a typical ASV analysis, a laboratorian uses a pipette to place 100 μL of anticoagulated whole blood into a tube containing a premeasured volume (2.9 mL) of the decomplexing reagent. The tube is placed in contact with the instrument electrode, and the analytical-cycle is started. The concentration of lead in the blood is available in units of μg/dL within 90 seconds.

During the first 60 seconds of the 90-second analysis time, a negative potential is applied to a mercury-coated graphite electrode. (Other electrode designs have also been used.) This process results in the lead (as Pb^{2+}) plating-out (or dissolving) into the mercury coat. Usually, the

potential is automatically and linearly swept back in a positive anodic direction. Other manufacturers may recommend functions other than a linear sweep (e.g., a square wave ASV). At a specific and characteristic voltage the lead is stripped from the electrode, a process that produces currents, the sum of which are proportional to the concentration of lead in the original sample. The samples may be prepared singularly or in a batch, and the analytical cycle is repeated for each sample. The operating parameters for analysis are normally established by the manufacturer and are set at installation. Each day the integration window, which must be symmetrically positioned about the lead peak, is verified and, if necessary, reset. This parameter is referred to as the integration set point.

Anticoagulants for ASV

Either heparin or EDTA may be used with the ASV method. Heparin requires no special procedures for analysis but may present a problem because of micro-clot formation, which makes the blood specimen non-analyzable. This problem most often occurs when blood specimens must be transferred from the collection site to a remote laboratory. EDTA is the preferred anticoagulant.

After the samples have been placed in tubes using a pipette, they should be incubated for 15-30 minutes before proceeding with the analysis. If standard evacuated glass tubes containing EDTA are used to collect blood, then the tubes must be at least half-full before the samples can be analyzed because, if the concentration of EDTA exceeds the usual 1.5 mg/mL, decomplexation of the lead is reduced, and the level of lead determined will be falsely low.

Calibration

When direct determination of lead levels in blood is performed by ASV (i.e., without acid digestion), calibration with aqueous lead standards is not possible, nor is it possible to use lead-spiked blood, since there are suitable differences between whole blood containing endogenous lead and whole blood spiked with inorganic lead. The instrument should be calibrated with blood-based materials traceable to the National Institutes of Standards and Technology (NIST) or to another well-characterized reference material. Blood-based calibrators may also be purchased commercially, prepared by the laboratory with the assistance of a reference laboratory, or they may be provided, along with the reagents, by the instrument manufacturer.

One reference laboratory with successful experience using ASV reports that calibration is most accurate if human blood, with endogenous lead levels that have been established by thermal ionization mass spectrometry (TI-MS), is used (J. Chisolm, MD, Kennedy Krieger Institute, personal communication, 1993). Certainly, other ASV reference laboratories[16] would also agree that success with determining blood lead levels depends on routinely checking instrument calibration with independently-validated blood-based reference materials, such as the Standard

Reference Material (SRM) 955a Lead in Blood from NIST, the CDC Blood Lead Laboratory Reference System (BLLRS) pools, or New York State's lyophilized reference material for blood lead determinations.

Another concern for ASV users is a potential interference from copper, which is oxidized or stripped at a potential close to that for lead.[16] Since copper is an essential element and is present in human serum in detectable amounts, the possibility exists for an interference, especially in populations where serum copper levels are elevated (e.g., in pregnant women). This interference is minimized by properly selecting the integration set point and using the currently recommended instrument parameters. Some laboratories use a strip-chart recorder to check for problems with copper interference.

Other electrochemical methods for measuring blood lead levels

Recent developments in analytical instrumentation have led to the development of prototype instruments for determining lead levels in blood by potentiometric stripping analysis (PSA), an electrochemical technique similar to ASV. Although not yet commercially promoted for measuring blood lead levels, PSA has been used successfully.[17,18] In addition, other electrochemical technologies are currently under development with the goal of providing a relatively inexpensive and portable means of screening children for lead poisoning.

Quality Assurance and Quality Control in the Clinical Lead Laboratory

Quality assurance and quality control can be loosely defined as those aspects of laboratory policy and practice which ensure that all test results are reported accurately. Although it is not possible to give a comprehensive description of all QA/QC practices, some aspects that are considered desirable for the clinical lead laboratory are given below. Under the 1988 Clinical Laboratory Improvement Act (CLIA) regulations, all clinical laboratories in the United States are required to document those aspects of tests considered of moderate or high complexity (Subpart K of 57 CFR 493), including the need to establish and verify method-performance specifications. These test aspects include accuracy, precision, reportable range, and analytical detection limit.

Control procedures

Under 1988 CLIA regulations, control procedures should be in place that monitor instrument stability and operator variance for all quantitative tests, and a daily QC procedure should be performed that includes at least two samples of different concentrations (normal and abnormal) to ensure the ongoing validity of test results (§493.1218). For blood lead measurements, the clinically relevant concentration range is 5 to 100 μg/dL of whole blood although confirmed blood lead concentrations above 70 μg/dL are rare. Most analytical methods are calibrated for a working range up to 60 μg/dL, with dilution required for specimens that are above the highest

calibration point. Most modern GFAAS methods have detection limits that are significantly better than 5 μg/dL and can, therefore, report test values of less than 5 μg/dL.

For daily QC monitoring, a variety of materials are available for blood lead measurements. (See Appendix C.2) We caution laboratories to verify independently each batch of daily QC materials against a certified reference material before they are used routinely. Any large deviation (1-2 μg/dL) from the stated target value warrants further investigation.

The acceptable range for routine daily QC should be no greater than that allowed for PT performance samples (i.e., $\pm$4 μg/dL or $\pm$10%, whichever is greater). Most laboratories experienced in analyzing blood lead levels should have no difficulty in achieving a day-to-day precision that is better than $\pm$ 4 μg/dL at values of 10 μg/dL. Therefore, CDC's Advisory Committee on Childhood Lead Poisoning Prevention recommends that, for investigative actions, laboratories set their internal QC limits to $\pm$2 μg/dL or $\pm$10%, whichever is greater.

Although 1988 CLIA regulations require a minimum of only two control concentrations (normal and abnormal) for blood lead measurement, the Advisory Committee also recommends that at least three levels of QC be employed at low ($\leq$ 10 μg/dL); intermediate (25-30 μg/dL) and high (40-60 μg/dL) calibration ranges for blood lead levels. The rationale for this recommendation is that most daily QC problems with blood lead measurements arise with the use of the high level control (> 40 μg/dL) because of sensitivity drift. If the blood lead level of a single high-level control (50 μg/dL) is out of the acceptable range, but the low-level control (10 μg/dL) is within range, then all test results in the batch (e.g., tray carousel) that are greater than the upper limit blood lead level of the low-level control (i.e., > 14 μg/dL) would have to be rejected and the specimen(s) reanalyzed. This reanalysis may create a problem if, for example, the rejected results were capillary specimens, and there was not enough sample remaining to perform a repeat analysis.

Under current regulatory guidelines, test results above the upper limit of the lead level of the low-level control can not be reported and the laboratory would request that another specimen be obtained. Had an intermediate control (30 μg/dL) been included in the run, the outcome might have been different. For example, an intermediate control might have been within acceptable limits, albeit with a low bias, but would have enabled the laboratory to report the results of testing a capillary sample (e.g., results between 14-34 μg/dL) without having to obtain another specimen.

Another reason for the tri-level approach is that with ASV analysis, when the electrode plate begins to thin, the higher blood lead values begin to loose accuracy (J. Chisolm, MD, Kennedy Krieger Institute, personal communication, 1993). Similarly, with GFAAS analysis, the most common problem is associated with unacceptable errors with high-level control values (i.e., values > 50 μg/dL).

Clinical lead laboratory approval and accreditation

The CLIA regulations of 1988 require that all clinical laboratories performing blood lead tests participate successfully in an approved PT program. Currently, five PT programs for proficiency in blood lead testing have been approved by HCFA for CLIA purposes (Appendix C.2). Laboratories participating in any one of three of these programs can also attain approval to perform blood lead testing from OSHA.

In addition to proficiency-testing requirements, many states require the mandatory reporting of blood lead test results. Some states have or are moving toward electronic reporting of all blood lead test results, whereas others require reporting elevated levels only. As states move to update their definitions of elevated blood lead tests results, many will require the mandatory reporting of all tests results, and the most efficient means for doing so is via electronic transmission. CDC's Advisory Committee supports the concept of electronic reporting of all blood lead test results by state since doing so will facilitate CDC's efforts in monitoring the incidence and prevalence of lead poisoning nationally. For additional information on electronic reporting of blood lead testing data, please contact your state health department or CDC.

Turnaround time for blood lead test results

Laboratory turnaround time for blood lead testing will depend on several factors, including the analytical method used, work-load fluctuations, and quality control protocols used. Although most public health laboratories only operate on a 9 a.m. to 5 p.m. weekday schedule, many private laboratories operate around the clock, and therefore, may be able to reduce turnaround time considerably. What is considered a reasonably turnaround time may differ for the different specimens that are analyzed. All laboratories recognize the need to allocate a higher priority to those specimens considered urgent (e.g., *stat* requests). However, capillary blood specimens, which are considered for screening purposes only, should be analyzed and reported to the requesting physician within 1 week of being received in the laboratory.

Venous blood specimens, which are more likely to be used for confirmatory follow-up purposes, should receive a higher priority than that allocated to a screening specimen. Confirmatory specimens should be analyzed and reported within 3 days of receipt in the laboratory. For *stat* requests, the laboratory should be able to reduce turnaround time to 24 hours or less. A *stat* request to determine a blood lead level from a capillary blood specimen is inappropriate because such a request implies that the result will be used to determine a course of medical management.

Reporting blood lead results

In the United States, blood lead test results are usually reported in units of micrograms per deciliter of whole blood (i.e., μg/dL or μg/100 mL. In most other countries, the international

system (SI) of units are preferred (i.e., micromoles per liter whole blood [μM or μmol/L]). To convert results in μg/dL to μM, multiply the former by 0.048; for example, 10 μg/dL - 0.48 μM.

A related aspect of reporting a blood lead value is the rounding-off of the result. The number of significant figures given is indicative of the precision of the analytical method. The convention in analytical chemistry is to report all the digits that are certain plus the first uncertain one.[19] The precision of most routine blood lead methods developed before the 1980s was only $\pm$ 1-2 μg/dL or greater. Thus, routine blood lead results were always rounded to the nearest integer for clinical purposes.

Now that the blood lead level of concern has been lowered from 25 μg/dL to 10 μg/dL of blood lead, analytical methods with improved precision have been developed for determining lower concentrations of lead in blood. For example, modern GFAAS methods can attain a between-run precision of approximately $\pm$ 0.3 μg/dL at low levels.[8] Therefore, with repeated analyses (n>3), blood lead test results at low levels could be reported to the first decimal with some confidence if such reporting were required (e.g., for research purposes). Of course, the laboratory would have to validate within-run and between-run precision to justify reporting results with such implied precision. For many routine blood lead testing methods, however, such precision is not justified for a single analysis; moreover, the clinical significance of such precision has not yet been established. Therefore, routine blood lead test results should still be rounded to the nearest integer.

Determining the Level of Lead in Urine

In the past, the primary purpose of determining lead levels in urine was to assess total lead excretion over a fixed period, usually 8 hours, as a function of the dose of calcium disodium ethylenedramine tetracetic acid ($CaNa_2EDTA$)given. Although using the lead mobilization or "provocation" test has decreased in recent years and demand for testing urinary lead levels has dropped, laboratories continue to receive specimens for analysis. Tests for lead levels on non-timed or "spot" urine specimens are considered to have little clinical value but are still used to monitor workers occupationally exposed to organolead compounds. Some guidance and recommendations for determining the level of lead in urine is provided below.

Recommended procedure for collecting urine samples

Collecting urine for a provocation test is almost always performed on an inpatient basis, and with very young children some additional difficulties occur in ensuring that the entire 8-hour specimen is collected without contamination from exogenous lead. A special commercially-available plastic pouch is taped over the child's genitalia to facilitate urine collection. However, it is the responsibility of the laboratory providing the analysis to ensure that all materials used to collect and transport urine specimens are lead-free.

For an 8-hour urine collection, the laboratory should provide a supply of primary urine collection containers (1-L volume) with caps, preferably plastic. Containers should be certified as lead-free, either by acid-washing them with 2% (v/v) nitric acid for 24 hours, followed by washing them with deionized water, or by selecting a small number from a batch, filling them with 2% nitric acid, and analyzing the leachate for lead. Either of these procedures should prevent the use of contaminated containers.

Transporting or shipping urine specimens to centralized laboratories

If the urine lead test is to be sent to a reference laboratory for analysis, then some consideration should be given to packaging and shipping the specimen. The laboratory performing the analysis should always be consulted because it may have specific requirements or employ a courier service. Generally, it is unnecessary and cumbersome to ship the entire urine specimen for analysis since the cost will be higher and risks for leakage greater. A 10-mL aliquot is usually sufficient for analysis, and this aliquot can be easily transported in commercially available plastic syringes designed specifically for transporting urine specimens.

There is no need to refrigerate urine specimens during transit since they are quite stable for several days. All urine collection materials should be provided by the testing laboratory and certified as lead-free.

Using additives as stabilizers

For determining lead levels in urine, some laboratories have proposed stabilizing the specimen with dilute nitric acid. However, the justification for acidification is weak, and addition of acid provides an opportunity to contaminate the specimen. Lead at high concentrations might precipitate out of solution at a pH of 6-7, but this is hardly the case with urine, where the pH is much lower. For this reason, it is unnecessary to add nitric acid to the specimen.

Analytical Methods for Determining Lead Levels in Urine

Most laboratories use GFAAS to determine lead levels in urine; ASV may also be used for the direct determination of urine lead levels, but analyzing urine by ASV requires different treatment than analyzing blood by ASV. When using GFAAS, the urine lead analysis can be more troublesome than that for blood lead because of the high inorganic salt content in urine and the lack of reliable urine reference materials with certified lead content at clinically relevant concentrations.

Graphite furnace AAS

For some furnace instruments, it may be possible to calibrate directly with aqueous lead standards.

If this is not possible, the best approach is to calibrate with matrix-matched standards (i.e., with lead-spiked urine containing nitric acid (2% v/v) and $NH_4H_2PO_4$ modifier). A donor should be sought who can provide urine samples containing little or no detectable lead. Typically, the calibation range for urinary lead is 0-60 μg/dL, as it is for blood lead. Because human urine can vary considerably, a 1+9 dilution with modifier/acid will help offset any potential interferences from other components present. As a rule of thumb, the analysis should always be approached by referring to the STPF concept.

Anodic stripping voltammetry

ASV can be used to determine urine lead levels, but several modifications are required for this analysis to be successful. First, calibrators specific for measuring urine lead levels should be used, and some laboratories recommend that nickel be incorporated into the supporting electrolyte/decomplexing solution to facilitate displacement of lead from EDTA (J. Chisolm, MD, Kennedy Krieger Institute, personal communication, 1993).

Quality Assurance and Quality Control

Few reliable reference materials exist specifically for QA/QC of urine lead measurements. NIST provides a bi-level Toxic Metals in Human Urine material (SRM 2670) that is certified for lead at an "abnormal" level of 109 μg/L, and a low level of 10 μg/L is given for informational purposes. However, this material has only limited application for clinical purposes, where typical urine concentrations range from 50 to 1000 μg/L. Using a 1+9 sample dilution, a linear calibration range up to 60 μg/L gives directly reportable concentrations of up to 600 μg/L. Clearly there is a need for reliably validated urine lead reference materials at concentrations between 100 and 600 μg/L. A list of urine control-material sources is provided in Appendix C.2.

Performance criteria for analyzing lead in urine

There are no established performance criteria for analyzing urine lead levels. Currently, no proficiency testing exists specifically for urine lead because the test is so rarely performed. Nontheless, it should be possible for analytical laboratories to achieve a level of performance of $\pm$ 60 μg/L at concentrations of <400 μg/L and $\pm$ 15% at concentrations above 400 μg/L. In fact, these were the performance standards expected for blood lead determinations before 1991, and they reflect the actual performance of a group of laboratories performing urine lead determinations using GFAAS, ASV, and MIBK-FAAS.[20]

Erythrocyte Protoporphyrin Test

The erythrocyte protoporphyrin (EP) test was used for many years throughout the United States as a screening test to identify children exposed to lead. In the 1991 edition of *Preventing Lead*

Poisoning Prevention in Young Children, CDC recommended that EP no longer be used as a screening test to detect lead-exposed children.[21] The justification for this recommendation was that the results of numerous studies showed the poor diagnostic sensitivity of EP for detecting blood lead levels at 10 μg/dL, and even at 25 μg/dL, coupled with an equally poor specificity.[22,23]

However, EP remains a valuable test in the medical management and follow-up care of children with confirmed elevated blood lead levels and as a screening test for iron deficiency. Blood lead-EP pairs are particularly useful in following long-term trends in lead absorption and in evaluating the question of internal redistribution of lead after chelation therapy.

Specimen collection

Either capillary or venous blood may be used for the EP test; however, blood specimens should be protected from prolonged exposure to light. For example, evacuated glass tubes can be wrapped in aluminum foil. The preferred anticoagulant for this test is EDTA, although heparinized blood may be also used. Instructions on capillary collection are given in Appendix C.2.

Analytical Methods for Determining EP

There are two principal methods for determining EP, acid-extraction and hematofluorometry. The extraction method is generally accepted as the reference method for EP and involves extracting protoporphyrin and other heme components from whole blood into a mixture of ethyl acetate and acetic acid and back-extracting the protoporphyrin into dilute hydrochloric acid. Quantitation is performed using a spectrofluorometer calibrated with protoporphyrin IX standards. A complete description of the extraction method is beyond the scope of this document; refer to the National Committee for Clinical Laboratory Standards' (NCCLS) document CP42-P on Erythrocyte Proroporphyrin testing.[24]

Hematofluorometry (HF) uses a small portable fluorometer dedicated specifically to measuring EP directly in whole blood as the zinc chelate, zinc protoporphyrin (ZPP). This instrument was once widely used in public health programs to screen children for lead exposure. Again, a complete description of the use and issues related to hematofluorometry is beyond the scope of this document; refer to the NCCLS document on EP testing mentioned above.

Millimolar Absorptivity (m) of Protoporphyrin IX

One issue yet to be completely resolved is continued widespread use of an incorrect millimolar absorptivity (m) value (241 L cm^{-1} $mmol^{-1}$)for protoporphyrin IX calibration standards. Since most hematofluorometers trace calibration back to the reference extraction method, the m issue affects hematofluorometry as well. Historically, the m value was thought to be 241

L cm^{-1} $mmol^{-1}$, but this value has been recently shown to be incorrect and the true value is 297 L cm^{-1} $mmol^{-1}$.[25] The impact of using the correct m value is a 19% decrease in all EP test results, including reference ranges. However, individual laboratories in the United States should not initiate any changes in calibration procedure unilaterally but should follow directives from the Health Resources and Services Administration (HRSA)-sponsored PT program for EP, operated by the Wisconsin State Laboratory of Hygiene, and from state PT programs for EP because such changes will have an impact on hematofluorometer calibration, target values for standards and controls, and reference ranges for EP.

Reference ranges for EP

In the 1985 edition of *Preventing Lead Poisoning in Young Children,*[26] CDC adopted an EP value of 35 μg/dL of whole blood as the upper limit of normal for screening children for lead exposure. This level corresponded to a blood lead level of 25 μg/dL. A simple adjustment of that value, correcting for the m error, results in a lower value of 28 μg/dL, which was adopted in the 1991 edition of *Preventing Lead Poisoning in Young Children*.[21] The NCCLS document on EP testing reports the correct upper limit of normal for EP, using m of 297 L cm^{-1} $mmol^{-1}$, to be 30 μg/dL, partly on the basis of reanalysis of the NHANES II data set, for which EP values were originally determined using an m value of 297 L cm^{-1} $mmol^{-1}$[24]. Thus, the CDC Advisory Committee, recognizing that EP has been plagued by historical inaccuracies in the analysis, recommends that the upper limit of normal for an EP test result is 30 μg/dL of whole blood. However, the Advisory Committee also reiterates that individual laboratories should follow guidance from PT program officials on the timing and manner in which this change in calibration should occur.

Standards and control materials for EP testing

The availability of standards and control materials for use in determining EP is limited. Pure protoporphyrin IX standards are available from only one source (Porphyrin Products, Logan, Utah). For extraction methods, lyophilized whole blood control materials are available from several sources and have been used as either control specimens or as secondary standards. Frozen whole-blood materials (human, bovine, or goat), if properly stored and protected from light, may also be used but are not commercially available. Hemolyzed or reconstituted lyophilized blood cannot be used with hematofluorometry because it requires the presence of intact red blood cells for correct quantitation. Since HF calibration is usually provided by the manufacturer, daily quality control is limited to testing liquid blood materials manufactured specifically for the hemotofluorometer (Appendix C.2).

Proficiency testing programs for EP

A limited number of PT programs are available for EP. They include the HRSA-sponsored program operated by the Wisconsin State Laboratory of Hygiene that is open to anyone and

several state-based PT programs (e.g., in New York, Pennsylvania, and New Jersey), where participation is required for in-state permit purposes. (See Appendix C.2). Although participation in a PT program for EP is not required by HCFA under the 1988 CLIA regulations, the Advisory Committee strongly recommends that all laboratories performing the analysis for EP participate successfully in such a program.

Miscellaneous Tests for Lead in Biological Fluids or Tissues

Lead Levels can be determined in a number of different tissues (teeth, hair, nails) and body fluids (breast milk, sweat, plasma). However, the clinical utility of such analyses is doubtful, and some tissues (e.g., hair, nails), may be so grossly comprised by contamination errors as to make their analysis totally unreliable. Tests to determine lead levels in teeth have proven useful in clinical research studies, [26,27] because they reflect cumulative lead exposure but are less valuable as routine clinical tests. Teeth may not always be available for specimens and may be compromised by inappropriate storage. The analysis is complicated by the fact that the tooth specimen must be digested under clean conditions before its lead content can be measured.

Digestion techniques are not routinely practiced by most clinical laboratories. No reference materials or controls are available to validate such analytical procedures and, given the regulatory requirements of CLIA, many laboratories capable of performing the analysis are reluctant to offer it because of such obvious difficulties. The same difficulties hold true for tests for lead levels in milk, hair, and nails.

References

1. Delves H.T. Blood collection for screening children for exposure to lead [[letter]. Clin Chem 1996;42:983-4.

2. Wang ST, Peter F. The stability of human blood lead in storage. J Anal Toxicol 1985;9:85-8.

3. Hessel DW. A simple and rapid quantitative determination of lead in blood. Atomic Absorption Newsletter 1968 7:55-6.

4. Delves HT. A micro-sampling method for the rapid determination of lead in blood by atomic absorption spectrophotometry. Analyst 1970;95:431-8.

5. Pruszkowska E, Carnick GR, Slavin W. Blood lead determination with the platform-furnace technique. Atomic Spectroscopy 1983 4:59-61

6. Shuttler IL, Delves HT. Determination of lead in blood by atomic absorption spectrometry with electrothermal atomisation. Analyst 1986;111:651-6.

7. Miller DT, Paschal DC, Gunter EW, Stroud PE, D'Angelo J. Determination of lead in blood using electrothermal atomization atomic-absorption spectrometry with a L'vov platform and matrix modifier. Analyst 1987;112:1701-4.

8. Parsons PJ, Slavin W. A rapid Zeeman graphite furnace atomic absorption spectrometric method for the determination of lead in blood. Spectrochimica Acta, Part B 1993; 48B:925-39.

9. Subramanian KS. Determination of lead in blood by graphite furnace atomic absorption spectrometry--a critique. Science of the Total Environment 1989;89:237-50.

10. Qiao H, Parsons PJ, Slavin W. Transferability of a simple method for blood lead determination using furnace atomic absorption spectrometry and continuum background correction. Clin Chem 1995;41:1451-4.

11. Flajnik CE, Shrader D. Determining lead in blood: evaluating deuterium and Zeeman background correction. American Clinical Laboratory 1994;13(9):45-6.

12. Helfrich JP. A workstation-based quick furnace method for the determination of lead in whole blood. American Clinical Laboratory 1994;13(4):14-5.

13. Carnrick GR, Slavin W. Modern graphic furnace AAS: part 1 and part 2. American Laboratory 1988;44B:188-97.

14. Slavin W. Graphite furnace AAS for biological materials. Science of the Total Environment 1988;71:17-35.

15. Campbell MJ, Delves HT. Accurate and precise determination of lead isotope ratios in clinical and environmental samples using inductively coupled plasma source mass spectrometry. J Anal Atomic Spectrom 1989;4:235-6.

16. Roda SM, Greenland RD, Bornschein RL, Hammond PB. Anodic stripping voltammetry procedure modified for improved accuracy of blood lead analysis. Clin Chem 1988;34:563-7.

17. Jagner D, Josefson M, Westerlund S, Aren K. Simultaneous determination of cadmium and lead in whole blood and serum by computerized potentiometric stripping analysis. Anal Chem 1981;53:1406-10.

18. Wang J, Tian B. Screen-printed stripping voltammetric/potentiometric electrodes for decentralized testing of trace lead. Anal Chem 1992;15:1706-9.

19. Miller JC, Miller JN. Statistics for analytical chemistry. Second edition. Chichester, United Kingdom: Ellis Horwood Ltd., 1988.

20. Parsons PJ, Jackson KW, Hughes M, Chace SW, Jenkins S. The influence of chemical modifiers on the atomization of lead from human urine by electrothermal atomic absorption spectrometry [abstract]. Clin Chem 1990;36:970-3.

21. Centers for Disease Control. Preventing lead poisoning in young children: a statement by the Centers for Disease Control. Atlanta, GA: Department of Health and Human Services, 1991.

22. McElvaine MD, Orbach HG, Binder S, Blanksma LA, Maes EF, Krieg RM. Evaluation of the erythrocyte protoporphyrin test as a screen for elevated blood lead levels, J Pediatr 1991;119:548-50.

23. Parsons PJ, Reilly AA, Hussain A. Observational study of erythrocyte protoporphyrin screening test for detecting low lead exposure in children: impact of lowering the blood lead action threshold. Clin Chem 1990;37:216-25.

24. National Committee for Clinical Laboratory Standards (NCCLS). Erythrocyte protoporphyrin: proposed guideline (C42-P). NCCLS document C42-P. Wayne, PA: NCCLS, 1995.

25. Gunter EW, Turner WE, Huff, DL. Investigation of protoporphyrin IX standard materials used in acid-extraction methods and a proposed correction for the millimolar absorptivity of protoporphyrin IX. Clin Chem 1989;35:1601-8.

26. Centers for Disease Control. Preventing lead poisoning in young children: a statement by the Centers for Disease Control. Atlanta, GA: Department of Health and Human Services, 1985.

27. Rabinowitz MB, Leviton A, Bellinger DC. Blood lead-tooth lead relationship among Boston children. Bull Environ Contam Toxicol 1989;43:485-92.

28. Rabinowitz MB, Leviton A, Bellinger D. Relationships between serial blood lead levels and exfoilated tooth dentin lead levels: models of tooth lead kinetics. Calcified Tissue International 1993;53:338-41.