Microsampling for Blood-Lead Analysis

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We detail the technique, procedure, and equipment required to properly obtain a micro-scale capillary (fingerstick) blood specimen for subsequent determination of its lead content. We divide the microsampling procedure into preparation of sampling equipment and subject, the fingerstick, micro blood collection devices, and transport and storage. The concept of a total-system approach to quality control for sampling and analysis is presented. For realistic quality control, the sampling component and the analysis component are inseparable. We describe types and sources of errors encountered. Most of the variability in the total system is associated with sampling.

Additional Keyphrases: toxicology • trace elements • environmental hazards • mass-screening technique • sample contamination

The prevalence of lead in our environment and its deleterious effects on the human body are well documented (1). A major source of lead ingested by children one to six years of age is leaded paint, found primarily on older, generally deteriorated dwelling units, in which the paint may be more readily accessible because it is chipped and peeling. About 600,000 children in the United States currently exhibit evidence of increased lead absorption, 6000 suffer permanent neurologic handicaps per year, and 200 young victims die annually. Massive screening programs have been initiated to detect children unduly exposed to lead. The most reliable indicator of undue exposure is the lead content of whole blood. Early detection permits the child's environment to be corrected before medical treatment is required.

The objective of a screening program is to rapidly sample a large population with a minimum of problems, time, and funds. Improved analytical instrumentation has facilitated the development of microanalytical methods for blood-lead analysis. The Delves cup modification (2-7), nonflame atomization (8, 9) atomic absorption spectroscopy and anodic stripping voltammetry (3, 10, 11) are the three principal microanalytical methods that are demonstrably amenable to routine high-volume analysis of 0.5-100 \(\mu\)l volumes of whole blood; such small volumes can be obtained by fingerstick microsampling.

Fingerstick techniques have been adapted for blood-lead determination to eliminate the problems associated with obtaining venous blood specimens from a small child. Fingerstick sampling is especially advantageous because sampling techniques can be quickly learned and applied by persons with no prior experience, trauma to the child is minimized, parental acceptance is greater, and special clinical facilities are not required. Fingerstick sampling is widely accepted in routine screening programs for the prevention of childhood lead poisoning.

The microsampling procedure is equally important with and inseparable from the microanalytical procedure that is used. Microanalyses are quite adequately described, but microsampling is only cursorily treated. Yet an improperly collected blood specimen yields meaningless data. Here, we discuss in detail microsampling for the subsequent determination of lead in blood.

The sampling component of the total sampling/analysis system may be conveniently subdivided into (a) preparation of the sampling equipment, (b) preparation of the subject, (c) the fingerstick, (d) specimen collection devices, and (e) transport and storage of the specimen. We also present the concept of a total-system approach to quality control, and discuss sources of error and their relative magnitude.

Microsampling
Preparation of Sampling Equipment

Strict quality control is essential. All equipment used for capillary sampling (lancet, swabs, dry gauzes, other cleaning solutions and materials, sample collection devices) should be subjected to frequent and rigorous batch testing before use, to ensure an inconsequential lead value by pre-established criteria of acceptability. Both field and laboratory personnel are responsible for ensuring that the equipment used is—and remains—reliable.
All materials and equipment must be handled as if a sterile specimen were being obtained. All supplies to be used for drawing the blood specimen must be individually packaged to avoid cross contamination. Such cross-contaminated materials contain an unknown and variable quantity of lead, and all blood-lead determinations performed on specimens collected with use of such contaminated supplies will be potentially erroneous. Individually packaged equipment will withstand a reasonable amount of rough handling without risk of contamination. Materials which have been opened, torn, or wet should be discarded.

Individually packaged dry gauzes are available in “peel-open” packages which, when opened, provide a lead-free surface on which to place other equipment. The alcohol swabs should be handled in such a manner that the surface coming into contact with the child’s finger is not touched by the technician.

Preparation of Subject

Before the sampling, the child’s hands should be washed, preferably with a scrub brush, thoroughly rinsed with warm water, and dried. Washing removes dirt and grime and stimulates bloodflow in the fingers.

The child may be held on a parent’s lap with his arm extended and supported at the elbow by the parent. The fingers and hand should be grasped in such a manner that the surface of the finger used for sampling cannot touch anything else, including other fingers (Figure 1). The surface generally used for sampling is a small area (about 1 cm²) on the lateral surface of the middle or ring finger; the hand should be held so that this surface points downward and the child’s palm is facing him. The sampling site is now vigorously cleaned.

Cleaning removes lead from the skin surface. Several different cleaning procedures and agents can be recommended for routine mass-screening situations. Kubasik et al. (8) obtain satisfactory blood specimens after a 1-min scrub with lead-free “pHisohex” soap followed by a rinse with de-ionized water. They attribute their satisfactory results to the fact that, upon puncturing the finger, the blood is isolated from the finger skin by the soap residue, minimizing contamination from the finger. Rosen (9) reports satisfactory results with an alcohol (isopropyl) scrub followed by a scrub with dilute (0.3 mol/liter) nitric acid and then with another alcohol scrub. Coleman (12) demonstrated the equivalency of a number of such cleaning agents: alcohol vs. water, alcohol followed by ethylenediaminetetraacetate (50 g/liter) vs. alcohol, nitric acid (3 ml/liter) vs. alcohol, and “Wool” brand hand soap followed by alcohol vs. alcohol. He concluded that use of nitric acid (3 ml/liter) followed by alcohol or of pHisoHex soap followed by alcohol may be superior to use of alcohol alone, but a scrub with alcohol is satisfactory. The crucial point may not be the choice of cleaning agent but rather the method of cleaning: a brisk mechanical scrubbing action for 30 s.

The cleaned finger area is wiped once with an untouched surface of a dry gauze sponge. The wipe is in the direction clean to unclean, leaving the sampling site completely dry.

Mitchell et al. (3) used a collodion spray to isolate the blood droplet from the skin. They compared three procedures: wiping the finger with an alcohol swab only; wiping the finger with an alcohol swab, drying, and applying collodion with an eye dropper onto the finger; and wiping the finger with alcohol, drying, and spraying collodion dissolved in ethyl ether from an aerosol can onto the finger. The collodion placed onto the finger dried in 15 s, the finger was lanced, and the blood sampled. After either collodion procedure, comparable blood lead values were obtained, but the lead values for fingers that had been subjected only to the alcohol wiping (not scrubbing) treatment were considerably higher; all values were compared with values for venous blood lead. Collodion applied as an aerosol spray is less liable to external contamination than collodion applied with an eye dropper; the former is therefore preferred.

Should the cleaned finger area come into contact with anything else before the sampling, the entire procedure must be repeated. Similarly, sampling equipment should be discarded if contaminated before use.

Fingerstick

The puncture produced by the lancet must yield a good blood flow; it should be made firmly and without hesitation. An off-center location on the fingertip is preferred because this location is less calloused than is the center of the finger tip and the stick appears to be less painful to the child. After the puncture, a drop of blood should be expressed and wiped off with a lead-free dry gauze in the direction clean...
to unclean. The child’s hand is held so that subsequent blood drops assume a pendant shape and have minimal contact with the skin.

To express the blood, the child’s fingers should be gently squeezed toward the puncture. Excessive squeezing must be avoided; if there is a poor flow of blood a new puncture is made. Berman et al. (13) reported that excessive squeezing may dilute the blood with tissue fluids, resulting in erroneously low values.

Blood that comes into contact with an unclean surface is never collected.

Blood-Collection Devices

Capillary tubes. Blood may be collected in glass capillary tubes (calibrated or uncalibrated) for subsequent storage and analysis. Capillary tubes may be heparinized to prevent clotting of the blood. Environmental Sciences Associates, Inc., 175 Bedford St., Burlington, Mass. 01803, supplies calibrated (100-μl) lead-free capillary tubes fitted with hard plastic caps with a protective sleeve over the inner core and with a plastic shock tube for storage and transport.

The ends of the opened capillary tube should be handled so that they contact nothing. This may be done by placing the tube onto a cradle so that the ends overhang the cradle.

The end of the tube is brought into contact with the pendant drop of blood; the blood flows into the tube via capillary action. The blood must fill the tube up to the calibration mark without air bubbles, as an exact volume is required for analysis. Air bubbles are completely avoided by holding the collecting end of the tube pointed slightly downward before collection. At no time should the tube contact the finger or be used to “scrape” blood from the finger. If either is done, the specimen should be discarded and the drawing repeated with a new tube.

If excess blood is collected into a calibrated tube, it must be immediately removed by gently flicking the tube rather than touching either end. After the blood is collected, the tube is tipped to position the blood column in the center of the tube, and the caps are placed onto the ends of the tube. The air plugs thus created will keep the blood in place during storage and transport.

Uncalibrated tubes, such as the Caraway or Natelson types, may also be used to collect blood specimens. After sufficient blood is collected, the tube is gently rocked or rotated 10 times to ensure thorough and complete mixing of the blood with the anticoagulant; a clotted sample collected in an uncalibrated container cannot be analyzed. “Critocaps,” “Plasticene,” “Plastic,” clay, or some other integral stopper may be used to cap the tube. Multiple caps supplied on a common stem are difficult to handle without contamination. Clay-type sealants are usually supplied in small cups or trays and the tubes plugged by pushing the ends into the clay. In addition to contamination through improper handling, after extended use such clay beds can cross contaminate the tubes and hence the blood. Also, many clays contain lead, and great care must be exercised to ensure that the blood does not come into contact with the clay. The lead in the clay may be chemically unavailable for contamination, but it can be a problem to properly remove the blood from the tube before the analysis.

Unopettes. “Unopette” (Becton-Dickinson, Rutherford, N. J. 07070) collection devices are manufactured in two general configurations: “Micro Lead Unopettes,” containing a predetermined volume of hemolyzing liquid to which a precise volume of blood is added, and “Capillectors,” containing a dry crystalline anticoagulant into which sufficient blood is collected for the analytical procedure used.

Micro Lead Unopettes (as developed by R. K. Simon and L. R. Bednarczyk, Delaware Medical Examiners Office, 200 S. Adams St., Wilmington, Del. 19801, in cooperation with H. W. Gerarde, Becton-Dickinson) consist of two parts (Figure 2): a sealed polyethylene reservoir containing 180 μl of dilute hemolyzing solution, and a glass capillary tube cut to contain 20 μl of blood. The capillary tube is protected from contamination by a plastic shield fitted over it.

The plastic capillary shield is wiped with an individually packaged alcohol swab to remove contaminants, and dried with a tissue. The pointed end of the shield is used to puncture the reservoir seal. The diluent must not contact the shield and none must be lost. With the shield remaining in position, the capillary tube may be laid down with little risk of contamination.

The blood is collected as described above. Flow into the capillary tube will automatically cease when the tube is completely filled; there must be no air bubbles. The outside of the tube is carefully cleaned.
with a lead-free tissue, care being taken not to draw any blood out of the tube. The tube is then inserted into the reservoir but not firmly seated. The shield is placed firmly over the other end of the capillary section. The positive pressure forces the blood into the diluent. The capillary is then firmly seated into the reservoir. The reservoir is gently squeezed several times to mix the blood and the diluent and to wash out the capillary tube. If the capillary tube has been contaminated or some blood or diluent lost, a new blood specimen must be collected with another Unopette.

The volume of blood collected is 20–50 μl and the diluent 100–200 μl; volumes are dictated by laboratory requirements. Various diluents have been used: “Triton X-100” (a hemolyzing agent); 50 ml of “Tergitol NPX” (a nonionic surfactant) per liter, in de-ionized distilled water; and dilute (30 ml/liter) acetic acid in de-ionized distilled water.

Capillectors consist only of the reservoir containing a crystalline anticoagulant and sealed with a polyethylene cap. They have been shown to be satisfactory for use in to mass screening programs (6, 9). To use, the cap is removed, exercising care not to contaminate the cap, as it will be replaced onto the reservoir and will come into direct contact with the blood. The blood may be collected with disposable cut-to-length (hence predetermined volume) glass capillary tubes and the blood dispensed into the reservoir; or the blood may be allowed to fall directly into the reservoir. The cap is replaced and the blood and the anticoagulant thoroughly mixed to prevent clotting.

Coleman (12) reports that use of the “dry” anticoagulant resulted in a larger-than-usual number of clotted blood specimens, presumably because of the difficulty of dissolving the anticoagulant in the blood. Hence, vigorous shaking is required.

Filter paper. Blood specimens may be collected by spotting a drop of blood onto filter paper (14–16). A drop of blood is allowed to flow uniformly onto a lead-free filter paper, and a given area of blood reproducibly corresponds to a specific volume. A disk of the paper containing the dried blood is subsequently punched out and used for analysis.

The filter paper is supplied individually prepackaged in a ribbed Petri dish or in a grooved polyethylene box or a bag. Punched holes, which serve a number of functions, may also be supplied. The blood spots collected must be at least this large (usually 0.25-inch diameter) to meet the requirements for analysis. The filter paper is held and touched only at the holes to prevent contamination of the rest of the paper. The number of holes also determines the number of spots of blood collected.

The pendant drop of blood to be collected is allowed to form on the child's finger. The drop should be sufficiently large to form the requisite spot size on the filter paper. Only one drop of blood should be used for each spot; two or more drops placed on the paper consecutively results in uneven flow; the first drop may already have partly dried, affecting the uniform spread of the remaining drops and leading to erroneous results. Also, the blood must not be smeared onto the paper, lest there be smearing, uneven distribution of the blood, and erroneous results.

Alternatively, the blood may be collected in a capillary tube and then spotted onto the paper. This ensures a sufficient volume of blood to form the spot.

The filter paper should be kept level until the blood has completely spread out. The wet spots must not contact anything. The dried spots may contact lead-free surfaces (inside of a bag) apparently without loss of lead.

Transport and Storage

Handling and storage of blood specimens subsequent to collection and before analysis depend on the chemical treatment afforded the specimen at the time of collection, the time and the distance between sampling and analysis, and the analytical method to be used.

Blood specimens are more stable if refrigerated (e.g., at 4 °C), but can be stored at room temperature. We recommend refrigeration of blood specimens collected with any of the Unopettes. No change in blood-lead values is reported for specimens stored for as long as 10 days at 4 °C (9). Blood specimens treated with sufficient heparin (500 int. units/ml of whole blood) may be maintained at room temperature for at least three weeks with no statistically significant change (because of adsorption) in lead concentration.

Improper preparation and handling of the blood specimen immediately after collection are prime sources of error. The blood and any anticoagulant used (if any) must be thoroughly mixed to ensure that sample integrity and homogeneity are maintained. If the specimen is maintained as a liquid, the container cap or seal must retain its integrity with time and temperature to prevent evaporation, spillage, or contamination.

The conditions to which the specimen will be subjected during storage and transport must be recognized and taken into account. Storage and packing requirements for specimens that are to be taken to a laboratory by the collectors are not so stringent as for specimens to be transported by freight, post, or air. For personal transport, the physical abuse to which the specimens will be subjected is generally known and no special shipping containers are required. Unopettes may be transported in a holding rack or in a plastic bag. Capillary tubes may be transported in shock tubes or taped between wooden tongue depressors. For long-distance transport, specially designed shipping containers are required that will securely hold the specimen container in place, withstand the expected physical abuse of routine handling, and meet other necessary requirements such as temperature control.

Complete kits, containing all the individually packaged equipment required to collect, store, and
transport blood specimens are available or can be
designed to meet the needs of the specific screening
program.

Training of Personnel
The procedure for obtaining a correct blood speci-
men is easily mastered, routine, and highly amena-
table to routine quality control. With proper training
under careful supervision, even an inexperienced vol-
unteer can be performing the entire procedure accu-
rately and routinely in a short time. Faulty tech-
nique can be minimized through sufficient emphasis
on the need to perform each aspect of the total task
correctly and completely.

Quality Control
Each batch of each and every item used must be
subjected to rigorous quality-control checks, to en-
sure an inconsequential contamination with lead.
This includes such items as lancets, cotton gauzes,
alcohol swabs, capillary tubes, caps, clay, Unettes,
and anticoagulant. Quality control should be per-
formed by the laboratory doing the blood lead analy-
ses and incorporated into the routine work load. All
items used for screening should pass first through the
laboratory to guarantee that quality checks have
been performed. It is the laboratory's responsibility
to ensure that the specimen is properly collected and
handled. Just because one batch of a given item
checks out satisfactorily does not guarantee that the
next batch of the same item from the same manufac-
turer can validly be used. Monitoring of each step
and quality control is a continuing process.

A total system consists of all the steps in handling
and analyzing a blood sample. An overall coefficient
of variation (CV) of ±10% is necessary and adequate
for diagnosing lead insult or lead poisoning. To ob-
tain and maintain this precision, all the precautions
described above are necessary.

Currently used analytical methods for blood-lead
determination can provide a CV of about ±4% at a
lead concentration of 40 µg/100 ml of whole blood, a
precision that is of little significance in determining
the total error of the system. The major errors arise in
making the specimen compatible, in all its transfer
steps, with the final analysis. The precision of per-
formance of a total system must be defined in terms
of thousands of actual specimens rather than 10 to 20
done under research conditions.

Matson and Griffin4 have defined the overall er-
errors of sampling, transport, preparation for
analysis, and analysis. Errors are assumed to possess
a gaussian distribution. They find that, for the total
system, most of the error is associated with sample

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