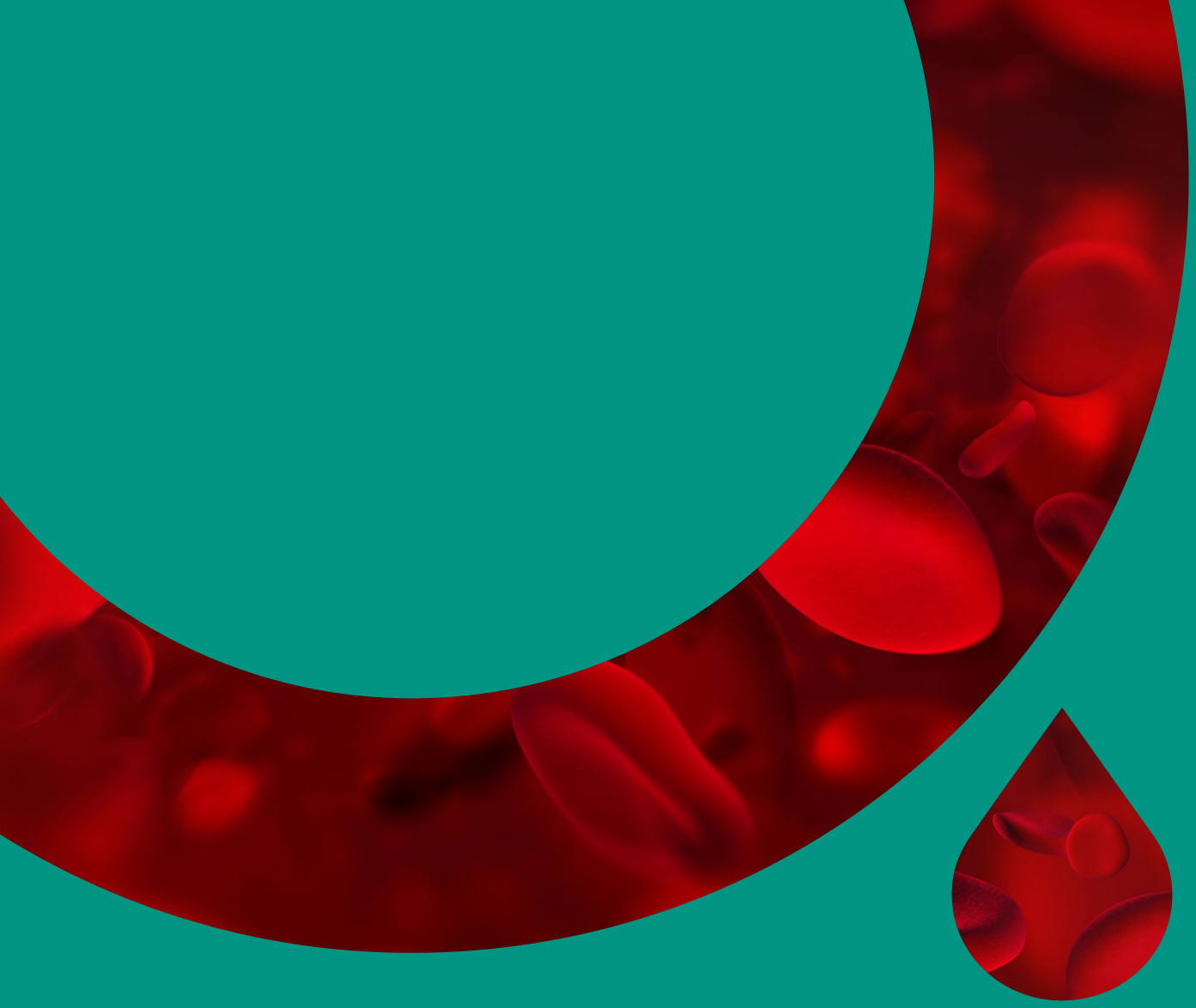

Brief guide to analytical methods for measuring lead in blood

Second edition

World Health Organization

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INVESTING IN OUR PLANET

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Abbreviations

AAS	atomic absorption spectrometry
ASV	anodic stripping voltammetry
CLIA	Clinical Laboratory Improvement Amendments
DALY	disability-adjusted life-year
dL	decilitre
ETAAS	electrothermal atomic absorption spectrometry
FAAS	flame atomic absorption spectrometry
GFAAS	graphite furnace atomic absorption spectrometry
HEPA	high-efficiency particulate air
ICP-MS	inductively coupled plasma mass spectrometry
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
L	litre
µg	microgram
µL	microlitre
µmol	micromole
mL	millilitre
WHO	World Health Organization

1. Purpose and scope

This document provides a brief overview of commonly used analytical methods for measuring the concentration of lead in blood. It is primarily aimed at informing public health personnel, scientific institutions and policy-makers who are not laboratory specialists but who may need to develop plans for conducting human biomonitoring studies, performing blood lead prevalence screening, and other public health actions related to the assessment of human exposure to lead.

For each analytical method there is a brief description of its characteristics, including its strengths and limitations. This document also highlights, for various types of applications and scenarios, the considerations when selecting an analytical method. It also describes considerations when deciding whether to establish a laboratory service for blood lead measurement, or whether to contract out. This document does not aim to provide a description of analytical methods and protocols or to make specific recommendations regarding methods or specific instruments. Detailed technical information on this subject is available elsewhere *(1)*.

2. Background

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular, musculoskeletal and renal systems. Long-term effects include increased risk of hypertension, ischaemic heart disease and renal disease. Children are particularly vulnerable to the neurotoxic effects of lead, and even low levels of exposure can impair cognitive development and cause behavioural disorders *(2)*. These effects can be lifelong *(3)*. As a consequence of the long-term effects on health, human exposure to lead in 2017 was estimated to account for 1.06 million deaths and the loss of 24.4 million disability-adjusted life-years (DALYs), with the highest burden falling on low- and middle-income countries *(4)*.

Despite recent reductions in the use of lead in petrol (gasoline), plumbing and solder, significant sources of exposure to lead remain. These include lead paint used on the interiors and exteriors of homes, schools and other buildings, and to paint toys, furniture, playground equipment and other articles with which children, in particular, can come into contact. Lead paint can give rise to lead-contaminated house dust, which is a major contributor to the total body burden of lead in children *(5)*. Other sources of lead exposure include the use of lead-containing traditional remedies; adulteration or contamination of food; lead ceramic glazes used in food containers; lead pipes and other lead-containing components in water distribution systems; and environmental contamination from industrial emissions, mining, disposal of electronic waste and use of ammunition.

Young children are particularly vulnerable to lead exposure because they absorb up to 50% of the ingested amount *(2)*. Acute lead poisoning from a single exposure is relatively rare and chronic poisoning is more common; however, the clinical features of poisoning are similar in both cases. The signs and symptoms are variable and may include anorexia, constipation, abdominal colic, anaemia, irritability, lethargy, muscle weakness, ataxia, tremors, convulsions and death *(2)*.

The clinical diagnosis of lead poisoning can be difficult when there is no clear history of exposure because poisoned individuals can be asymptomatic, and signs and symptoms, when they are present, are relatively non-specific. The measurement of the blood lead concentration is the only reliable way to diagnose lead-exposed individuals and, therefore, it plays an essential role in the identification and management of lead poisoning and in the assessment of occupational and environmental lead exposure *(6)*.

Although other human tissues and fluids, such as hair, teeth, bone and urine, also reflect lead exposure, the quantification of lead in whole blood has gained wide acceptance as the most useful tool for screening and diagnostic testing *(1, 7)*. This is because there are well established methods for sample collection and analysis and there is a large body of information linking blood lead concentrations with clinical effects and treatment outcomes. Moreover, validated analytical methods and reliable blood quality control and reference materials are available.

3. Measurement of lead in blood

The most common reasons for measuring the blood lead concentration are as follows: to determine the need for the active management of lead exposure, for example

- identification of, and removal from, the source of exposure, or chelation therapy;
- to determine the effectiveness of risk mitigation measures;
- as part of a health screening or surveillance programme to identify lead-exposed children;
- for exposure and risk assessment, for example a prevalence study of lead exposure related to lead paint or other sources;
- for occupational monitoring.

The purpose of the analysis may have a bearing on the choice of analytical method, as discussed in section 7.

3.1 Units

The commonly used units for reporting blood lead concentration are micrograms of lead per decilitre of blood (µg/dL), micrograms per litre (µg/L) and micromoles per litre (µmol/L). The conversion factor between mass and molar units is the atomic mass of lead: 207.19. When making the conversion it is important to be careful about the denominator – that is, decilitres or litres.

- For conversion from mass to molar units the value should be divided by the atomic mass.
- For conversion from molar to mass units the value should be multiplied by the atomic mass.

Some example conversions are given in Table 1.

Table 1. Conversions between mass and molar units for blood lead concentration

µg/dL	µg/L	µmol/L
5	50	0.24
45	450	2.17
70	700	3.38

3.2 Blood sampling

In setting up a system for sample collection and transfer to the laboratory, certain requirements must be met to ensure that the correct sample is collected and that it is delivered to the laboratory in good condition.

The pervasiveness of lead means that the risk of sample contamination is high, and contamination can occur at each stage: sample collection, storage, transport and manipulation. A clean location should, therefore, be identified for sample collection and storage – this is particularly important if samples are being collected in the field. The puncture site must be thoroughly cleansed before blood is taken, to remove any surface contamination with lead. Sample collection equipment and containers, including needles and caps, must be clean. Ideally, they should be certified for blood lead testing or at least they should be pre-screened to measure their lead content *(1, 8)*.

The blood lead concentration can be measured in venous or capillary blood samples. Venous sampling is a more invasive technique but allows more accurate quantification of body burden. Analysing venous blood for lead is preferred for confirmation of exposure, diagnosis, decisions on the medical management of lead poisoning and prognosis. Capillary blood sampling, involving a finger (or heel prick for babies), is relatively easy and quick and is less invasive but may be affected by contamination (giving false positives). Capillary blood is acceptable for initial screening purposes *(1)*. As the finger or heel are highly likely to be in contact with lead-contaminated media, thorough cleansing of the sampling site is particularly important.

Trained health care staff are required for sample collection. Universal precautions for preventing bloodborne transmission of infection must be observed for all types of blood sampling. General guidance on best practice and on equipment needs for blood sampling is provided in the World Health Organization (WHO) publication *Guidelines on drawing blood: best practices in phlebotomy (9).*

4. Analytical methods used to measure lead in blood

The measurement of the blood lead concentration can be carried out using laboratory methods and point-of-care or field-testing methods. These methods differ significantly in their analytical capacities (limits of detection, accuracy and precision), costs (purchase and maintenance costs, laboratory infrastructure required, reagents and supplies) and technical requirements (sample preparation, calibration and skilled personnel). These factors, taken in conjunction with the setting and resources of the laboratory, will influence the decision about the choice of method. Additional information is provided in Table 2 below.

4.1 Laboratory methods

The most commonly used reference methods for determining the blood lead concentration are electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS). Flame atomic absorption spectrometry (FAAS) can also be used but has some limitations. Other methods less commonly used clinically, such as inductively coupled plasma optical emission spectrometry, are not described here.

4.1.1 Atomic absorption spectrometry (AAS)

The principle behind atomic absorption spectrometry (AAS) is the interaction between outer-shell electrons of free, gaseous, uncharged atoms and ultraviolet or visible light generated from the element to be measured. A lamp with a hollow cathode coated with lead emits light from excited lead atoms with characteristic wavelengths that can be absorbed by lead atoms in the sample. The light passes through the atomized sample and some energy is absorbed by the lead atoms, reducing the amount transmitted to the detector. The amount of light absorbed (or absorbance) is related in a linear fashion to the concentration of the analyte in the sample *(10)*.

To conduct an AAS measurement, the lead-containing sample must first be introduced into the instrument so as to generate ground-state atoms in the gas phase within the optical path of the instrument. This process, called atomization, can be achieved using either a flame (flame atomic absorption spectrometry, or FAAS) or an electrothermal device (electrothermal atomic absorption spectrometry, or ETAAS) *(11)*. Although FAAS and ETAAS have similar detection principles, they differ greatly in their applicability to the measurement of lead in blood (for example with regard to limits of detection, sample size and complexity of sample preparation).

4.1.2 Flame atomic absorption spectrometry (FAAS)

FAAS typically uses an air-acetylene flame to atomize lead at temperatures in the order of 2100–2400 °C *(10)*.

The sample size required and limit of detection using FAAS depends on sample preparation and the method used to present the sample to the flame for atomization. Typically FAAS requires millilitre (mL) sample volumes; however, Delves cup AAS, an older microsampling method, has enabled the use of 50–100 microlitre (µL) sample sizes. A limit of detection around 5 µg/dL can be obtained *(12, 13)*. This is no longer useful for screening in populations where low background blood lead concentrations are less than 5 µg/dL.

Some laboratories may choose to analyse blood for lead in duplicate or even triplicate to reduce analytical uncertainty *(8).* This increases the turnaround time for blood lead analyses. At the very least, elevated results for blood lead should be confirmed with a second analysis on another aliquot of blood *(1).*

FAAS has largely been superseded by ETAAS, which can determine much lower blood lead concentrations on smaller samples *(8, 10, 11, 14)*.

4.1.3 Electrothermal atomic absorption spectrometry (ETAAS) or graphite furnace atomic absorption spectrometry (GFAAS)

Most ETAAS systems use an electrically heated graphite tube to pyrolyse the blood matrix and atomize the lead, so this method is also known as graphite furnace atomic absorption spectrometry (GFAAS). The pyrolysed sample is heated to a temperature of ~1700 °C to atomize lead *(11).*

ETAAS measurements can be subject to significant interference from light scattering and molecular absorption by matrix components, but this can be mitigated by using various approaches, including the use of chemical modifiers and Zeeman background correction techniques *(1, 10, 13)*. ETAAS devices must be operated by trained laboratory technicians.

ETAAS instruments are widely available. They require only small sample volumes, typically 50–100 µL *(10, 11).* The limit of detection for lead is in the low parts per billion range (as measured in µg/L); for example, a method has been described with a detection limit of 0.65 µg/L (0.065 µg/dL) *(15)*. These instruments can be left to run unattended. The use of autosamplers increases precision, and sample throughput is approximately one sample every two to four minutes *(8, 11, 14)*. The initial instrument cost is intermediate, but maintenance and consumable costs are significant *(11)*.

4.1.4 Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) is a multi-element technique that uses an inductively coupled plasma (a very high-temperature ionized gas composed of electrons and positively charged ions) source to dissociate the sample into its constituent atoms or ions. The ions are extracted from the plasma and passed into a mass spectrometer, where they are separated and measured based on their mass-to-charge ratio. The efficiency of the inductively coupled plasma in producing ions from the atoms of interest in the aerosolized sample, coupled with the high selectivity of the mass spectrometer (typically a quadrupole mass analyser which filters the ions), the high amplification of ionic signals striking the detector, and the low background noise of the detector, provides very low instrumental detection limits (parts per billion to low parts per trillion for most elements) *(10, 11)*. A method has been described that gives a limit of detection of 0.15 µg/L (0.015 µg/dL) *(16)*, which meets clinical and human biomonitoring needs.

ICP-MS devices usually require highly skilled laboratory technicians for operation at the highest standards *(1, 11).*

While other methods can measure only one or a few elements at a time, ICP-MS can measure multiple elements from a single blood sample as small as 50–100 µL. This would be an important consideration for laboratories wishing to measure a number of elements in addition to lead. Some ICP-MS instruments also enable determination of the isotope ratio of the lead present in a sample, which may make it possible to identify whether the lead came from a particular source.

4.2 Anodic stripping voltammetry (ASV)

4.2.1 ASV technique

Anodic stripping voltammetry (ASV) is an electrochemical technique for measuring the blood lead concentration. To conduct an ASV measurement, the blood sample must first be treated to release the lead in ionic form from red blood cells and proteins. A reference electrode

and a thin-film mercury graphite electrode are placed in the treated blood sample. A negative potential is then applied to the mercury electrode for a period of time, which causes lead and other cations present in the sample to concentrate on the surface of the negatively charged mercury electrode. The direction of the potential is then reversed to give an increasingly larger potential over several minutes. As the voltage reaches a specific and characteristic voltage for lead, all the lead ions are released (stripped) from the electrode, thereby producing a current that can be measured. The current produced is proportional to the number of lead ions released and can be compared with calibration solutions to determine the lead concentration in the sample *(1, 10).*

Laboratory-based ASV is no longer commonly used for the measurement of the blood lead concentration. There are, however, portable ASV instruments that can be used at point of care and in the field, and that may also be set up in a laboratory.

4.2.2 Portable ASV device

Portable instruments using disposable screen-printed electrodes are available for on-site testing of blood lead concentrations. This form of testing performed outside a laboratory setting is known as point-of-care or near-patient testing, or a rapid diagnostic or rapid screening test. Portable ASV instruments can be used for screening for lead exposure, for example in child health clinics and in mobile clinics, and for carrying out epidemiological studies or outbreak response activities at locations where transport of blood samples to an appropriate reference laboratory is difficult *(17, 18)*.

As mentioned in section 3.2, it is particularly important to take care to avoid sample contamination when a portable device is used outside a laboratory setting and when capillary blood samples are being collected. It is normally recommended that elevated blood lead concentrations (≥ 5 µg/dL) measured on capillary blood with portable instruments are confirmed by repeated analysis in a certified laboratory *(1, 17)*. As the result from the portable instrument is delivered quickly, it is possible to take a venous sample straight away for confirmatory laboratory analysis.

The United States Centers for Disease Control and Prevention, in collaboration with a private company, has developed a portable ASV instrument called LeadCare II, which is commercially available. This instrument does not require skilled laboratory personnel for its operation and is classified by the United States Food and Drug Administration as CLIA waived[1] (that is, it has a lower level of complexity with fewer regulatory requirements).

LeadCare II allows the determination of blood lead concentrations within three minutes using a 50 µL sample of capillary (fingertip) blood or venous blood. The single-use sensor, sample container, reagents and calibration equipment are provided as disposable units that are precalibrated by the manufacturer. The reportable range of blood lead concentrations is 3.3–65 µg/dL *(1, 17).* Blood lead concentrations higher than the operating range have been measured successfully using appropriate sample dilution techniques *(19).*

Studies have shown good correlation between analyses carried out using the LeadCare II device and those carried out using GFAAS *(17).* Note, however, that artificially low blood lead concentrations have been reported on venous samples collected in certain vacutainers owing to a reaction between a chemical in the rubber stopper and lead in the sample *(20).* It is advisable therefore to check with the instrument manufacturer about sample containers.

1 The United States Food and Drug Administration is responsible for the categorization of commercially marketed in vitro diagnostic tests into one of three CLIA (Clinical Laboratory Improvement Amendments of 1988) regulatory categories based on their potential for risk to public health: tests of high complexity; tests of moderate complexity; and waived tests.

There may be other brands of portable ASV, but these could not be identified at the time of writing.

4.3 Choosing the appropriate instrumentation

The choice of instrumentation depends on the reason for analysis (for example, exposure assessment, diagnosis or screening), the number of samples to be tested, cost limitations, the need for precise measurement, and the availability of trained personnel and analytical equipment.

Laboratory-based methods require trained staff, though the level of training and skill required varies with the instrumentation. FAAS systems are usually relatively easy to set up and run but do require some laboratory expertise. This is particularly true if there is a need to measure lower blood lead concentrations, as more sophisticated protocols will be needed. ETAAS systems are somewhat more difficult to set up and maintain and require laboratory expertise. ICP-MS generally requires highly skilled laboratory personnel to achieve superior results and reliable, high-quality data.

Point-of-care methods are very simple to use and do not require trained laboratory personnel. When using these methods in the field there is a need to consider and mitigate environmental conditions that could interfere with their reliability. These include environmental contamination risks and the need to find a clean space for sampling and testing, the availability of water and soap for adequately cleansing the skin of test subjects, temperature requirements of the analysers, and the availability and quality of the electricity supply.

The various characteristics of the different analytical methods are summarized in Table 2.

Table 2. Overview of analytical methods for blood lead measurement

Method	Strengths	Limitations
Flame atomic absorption spectrometry (FAAS) *(11, 12, 14, 15)*	• Short analysis time (seconds) • Relatively easy to use • Relatively few interferences • Relatively low capital and running costs	• Large sample size usually needed • Relatively high detection limit (5 µg/dL) • Cannot be left unattended (flammable gas)
Electrothermal atomic absorption spectrometry (ETAAS) *(8, 11, 14, 15)*	• Low detection limit (< 1 µg/dL) • Can analyse small samples (50–100 µL) • Can be fitted with autosampler so multiple samples can be processed • Well documented applications • May be left unattended • No need for sample preparation	• Limited analytical working range • Requires some laboratory expertise • Longer analysis time so low sample throughput
Inductively coupled plasma mass spectrometry (ICPMS) *(10, 11, 14, 16)*	• Very low limit of detection (0.02 µg/dL) • Can analyse small samples (50–100 µL) • Very fast analysis time (< 1 minute) • Wide analytical working range • Multi-element capabilities and can be economical if used for large sample runs • Potential to perform isotopic ratio analyses with some forms of ICP-MS, which may help to identify the source of the lead	• High purchase and running costs • Requires highly skilled laboratory staff • Analysis of large number of samples is cheaper than ETAAS
Portable ASV *(1, 17)*	• Small sample size (50 µL) • Can be used at non-laboratory sites • Uses finger prick (capillary sample), though venous samples can also be used • Simple to use, does not require skilled laboratory personnel • Low purchase and running costs • Rapid results • Has comparable accuracy with laboratory-based methods	• Limited analytical working range • Levels above 5 µg/dL should be confirmed by a high-complexity laboratory method • High risk of sample contamination • Risk of low-biased results on venous blood collected with certain evacuated blood tubes *(20)*

5. Choosing a laboratory

As described above, the blood lead concentration can be measured using a laboratory method or a point-of-care device. Point-of-care devices are useful for screening purposes. A point-of-care device has also been used to guide the management of poisoning in a low-resource setting where there was no ready access to a proficient laboratory *(18)*. In this case, results were validated using a split sample protocol, whereby a proportion of samples were sent to a reference laboratory and the results obtained were compared *(19)*.

Analysis in a reference laboratory using a high-complexity method is preferred for most purposes. There are several considerations when selecting a laboratory. Of key importance is the quality of the service provided. Using a laboratory that is accredited by a national accreditation body or that participates in an external quality assessment or proficiency testing programme for blood lead analysis (see section 6.2) will give confidence in the accuracy and reliability of the analytical results obtained. This is necessary if results are to be compared over time or with different geographical areas. More information on laboratory quality, including accreditation, is provided in section 6.

Another factor is the instrumentation that the laboratory uses and whether analyses can be provided to the required limit of detection with the necessary accuracy and precision. If there is interest in using isotope ratio analysis to try and identify an environmental source of exposure, then a laboratory that can offer analysis by ICP-MS and is experienced in isotope ratio measurements is needed.

Other important considerations include:

- the capacity to handle the number of samples required
- the costs of analyses, including shipping costs
- the turnaround time.

5.1 Finding a laboratory

Information on accredited laboratories can usually be found on the website of the national or state accreditation body. Contact details of national accreditation bodies are given on the website[2] of the International Laboratory Accreditation Cooperation (ILAC), which lists those bodies that are signatories to the ILAC Mutual Recognition Arrangement. The website also gives information on regional accreditation cooperation bodies.

If an accredited laboratory is not available, then a laboratory that can demonstrate compliance with a quality management system and that regularly participates in a proficiency testing scheme can also deliver reliable and accurate analyses.

If there is no laboratory in a country that operates to the required standard, it may be necessary to send samples to a laboratory abroad. In the longer term, however, consideration should be given to establishing at least one accredited laboratory service in the country.

2 https://ilac.org/signatory-search.

5.2 Establishing a laboratory service to measure blood lead concentrations

Establishing a laboratory service requires a significant investment of resources. Some points to consider when deciding whether to go ahead are given below.

- Is there a sufficient workload to justify setting up the service?
- Is there another laboratory in the country or abroad that is already providing this service at a reasonable cost and within an acceptable time frame?
- Is there an existing laboratory that can add blood lead analysis to its services?
- What type of instrumentation is needed for the purposes of the laboratory (such as ETAAS, ICP-MS)?
- Is the necessary analytical equipment already available or must it be purchased?
- Is existing equipment still supported for maintenance and repair? If not, then laboratory personnel will need additional skills to keep the equipment operational to an adequate standard.
- Are there a sufficient number of adequately trained laboratory personnel to operate the selected instrumentation?
- Are there sufficient funds for the purchase of equipment, its installation, and maintenance and operating costs, including purchase of certified reference materials and replacement of consumables such as lamps, tubes and gases?
- Are there suitable premises for the laboratory with a reliable and consistent power and water supply? Can an existing building be modified or is it necessary to build a new laboratory?
- Will the laboratory seek accreditation or other means of demonstrating compliance with a quality management system, and are resources available to support this?

6. Important aspects of laboratory practice

In analytical toxicology, even the most sophisticated and accurate equipment will provide incorrect results if care has not been taken to prevent sample contamination, the samples have not been appropriately handled and stored, the equipment has not been used correctly, or analytical protocols have not been followed. These potential problems can be minimized by the implementation of an adequate quality management system, which is discussed below. In addition, several organizations *(1, 8)* and government agencies have developed good laboratory practice guidance for blood lead testing *(21)*.

6.1 Preventing external contamination

As mentioned earlier, lead is pervasive and can contaminate samples in numerous ways. Contamination can occur during sample collection, sample storage and transport, and sample manipulation *(8)*. The quality of sample collection and handling is therefore crucial for the accurate determination of lead in blood. As discussed in section 3.2, precautions are needed when samples are collected, including the use of pre-screened or certified sampling equipment. As aliquoting samples in the laboratory can introduce the risk of contamination, it is best to analyse the samples directly from the collection tubes. If aliquoting is required, then it is advisable to use pre-screened plastic tubes or cryotubes without acid washing. Acid washing can, if not carried out in the correct manner, cause unintended lead contamination.[3]

If the laboratory also analyses environmental samples, this should be carried out in a separate area from analysis of biological samples. Sample preparation should be performed in a clean environment, dedicated to blood lead analyses. If possible, samples should be prepared in a laminar flow biological safety cabinet (such as a Class II, Type B2 cabinet).[4] If this is not available, the possibility of airborne contamination should be considered, and periodic controls performed to detect it. Measures should, in any case, be taken to try and minimize the amount of air particulates (such as dust or outdoor particulates) in the laboratory and in the area where open sample tubes will be located during analysis. Frequent wet mopping of floors and wet wiping of laboratory surfaces can help minimize this source of contamination. Instrument autosamplers should be protected with dust covers, and autosampler vials screened for lead contamination in order to identify a supplier of lead-free vials *(8)*.

Contamination risks can be significantly reduced by the application of adequate quality assurance measures *(1, 8)*. Specific protocols are available for the different analytical methods, including from manufacturers and standardization agencies, and these should be strictly followed.

3 Jan Kuta, personal communication, April 2020.

4 Class II, Type B2 cabinets provide protection to the sample, the user and the environment. In these cabinets, air is filtered with a high-efficiency particulate air (HEPA) filter and is pumped into the cubicle at a minimum inflow velocity of 0.5 metres per second. Inside the cubicle, air circulates in a laminar flow and is then pumped out with no air recirculation after a second HEPA-filtering step.

6.2 Quality assurance and quality control

Quality assurance and quality control are components of a quality management system. Quality management involves the integration of all aspects of laboratory operation, including the organizational structure, processes, procedures and resources, in order to ensure that the service provided to users is of high quality and laboratory results are reliable and reproducible *(22)*. WHO has published guidance and training materials on laboratory quality management *(22, 23)*.

Quality assurance is concerned with processes and procedures. It covers the utilization of scientifically and technically sound practices for laboratory investigations, including the selection, collection, storage and transport of specimens and the recording, reporting and interpretation of results. It also refers to training and management designed to improve the reliability of investigations. Quality assurance includes the initial assessment of an analytical method as to its practicability and trueness, which includes linearity, specificity, recovery, calibration standards, blanks, limits of detection and limits of quantitation and robustness *(24)*.

Quality control refers to the control of errors in the performance of tests and verification of the test results. It has two components: internal quality control and external quality assessment.

6.2.1 Internal quality control

Internal quality control is a set of procedures used by the staff of a laboratory for continuously assessing results as they are produced to determine whether they are accurate, precise and, therefore, reliable enough to be released. An example of a quality control measure is the daily measurement of control samples of blood with a well characterized lead content to check the performance of the analytical method *(1, 8)*. Another example is the use of reference materials, also known as reference standards, to validate and calibrate the analytical method and to create quality control charts. Certified reference materials for blood lead analysis are available.

The standard operating procedure for a test should normally include a description of quality control measures.

6.2.2 External quality assessment

External quality assessment is a system for objectively checking laboratory performance using an external agency. It involves the laboratory being sent “blind” test samples in which the quantity of lead is unknown. The analytical results are then compared against the actual lead concentrations, which are not revealed until after the analyses have been completed. The results may also be compared against those of other laboratories participating in the scheme as a means of proficiency testing *(25)*. External quality assessment schemes normally involve several test cycles each year.

There are a number of external quality assessment or proficiency testing schemes for lead in blood. A selection is listed in the Annex.

6.3 Quality standards, certification and accreditation

Quality standards, certification and accreditation are important measures for ensuring and demonstrating laboratory quality *(22)*.

A standards document – established by consensus and approved by a recognized body – provides, for common and repeated use, guidelines or characteristics for activities or their results, aimed at the achievement of the optimal degree of order in a given context. Standards may be developed nationally or internationally *(22)*. Examples of international standards relevant to laboratories that measure lead in blood are those developed by the Clinical and Laboratory Standards Institute *(1)* and the International

Organization for Standardization (ISO) *(26, 27)*. In the case of a laboratory measuring blood lead concentrations, two relevant ISO standards are ISO 15189:2012 on requirements for quality and competence in medical laboratories, and ISO/IEC 17025:2017 on general requirements for the competence of testing and calibration laboratories *(26, 27)*.

Certification is the procedure by which an independent body gives written assurance that a process or service conforms to specific requirements. This involves inspection of the laboratory by representatives from a certification body, who are looking for evidence of compliance with standards, policies, procedures, requirements and regulations. The main assessment is for the physical presence of procedures and documents *(22)*.

Accreditation is the procedure by which an authoritative body gives formal recognition that the laboratory is competent to carry out specific tasks, for example quantification of lead in blood. In this case the laboratory is inspected by representatives from an accreditation body who, in addition to looking for evidence of compliance with standards, policies, procedures, requirements and regulations, also assess competency by observing the laboratory staff. The accreditation body may also set the standards with which the laboratory must comply, for example ISO 17025:2017.

7. Scenarios

This section presents some typical scenarios in which blood lead measurements are required, with pointers to some of the considerations that will influence the choice of analytical method.

7.1 Management of lead poisoning

Determination of the blood lead concentration is crucial for the diagnosis of suspected cases of lead poisoning and to guide subsequent management, such as the use of chelation therapy. Measurement of the blood lead concentration may be required for a single individual (for example, a child who has ingested a fishing weight or lead paint flakes, or an adult who has been using a lead-containing traditional medicine) or for a group of people (for example, lead poisoning outbreaks caused by environmental contamination from mining or smelting).

For this purpose, the rapid availability of results is an important requirement, in particular if exposure levels are acutely life threatening. A low limit of quantification is generally not required, though accuracy and precision are important. Laboratory-based methods are therefore preferred. Using a portable ASV device may be helpful for rapid patient triage in the case of a mass poisoning; however, positive results should be confirmed by laboratory analysis using a method based on either ETAAS or ICP-MS.

There is experience in using portable ASV instrumentation to support the management of mass poisoning in a low-resource setting where laboratory-based analyses were not available. In this case care was taken to avoid sample contamination, analyses were carried out on venous samples, and a proportion of blood samples were regularly sent to a reference laboratory abroad to validate the results *(18)*. As the blood lead concentrations were generally high, and above the operating range of the device used, a method was developed and validated for diluting the blood samples before analysis *(19)*.

7.2 Exposure and risk assessment

Determination of blood lead concentrations may be required as part of a health risk assessment for a population that is possibly exposed to lead, such as a community living near a lead-processing factory. Health risk assessments include an exposure assessment step to measure the magnitude, frequency and duration of exposure to lead, along with the number and characteristics of the population exposed. Guidance on carrying out such studies has been published by the United States Centers for Disease Control and Prevention *(28).* Although various biomarkers can be used to evaluate human exposure to lead, the blood lead concentration is the best validated and most widely used *(7)*.

Some countries carry out periodic national human biomonitoring surveys to assess exposure to a range of chemicals, including lead. Examples are those carried out by Canada, Germany and the United States of America. In addition, a European Union human biomonitoring project is under way *(29)*. Over the course of several rounds of surveys, blood lead concentrations have been shown to be declining at the population level. In the United States, for example, the most recent geometric mean value is 1.12 µg/dL (in 2009/2010) and the 95th percentile is 3.34 µg/dL, compared with 1.66 µg/dL and 5.0 µg/dL respectively in 1999/2000 *(30)*. In Canada the most recent geometric mean is 0.93 µg/dL (in 2016/2017) and the 95th percentile is 2.5 µg/dL, compared with 1.2 µg/dL and 3.2 µg/dL respectively in 2009/2011 *(31)*.

For exposure assessment, analytical methods are required with a high level of accuracy and precision (to enable accurate comparison of results with future or past measurements) and a low limit of detection (to determine low levels of exposure), such as ETAAS or ICP-MS. ICP-MS also offers the possibility of using isotope ratio analysis to identify the environmental source of exposure *(32).*

7.3 Screening and surveillance

A number of national public health agencies, for example in France and the United States, make recommendations regarding screening and surveillance of children for lead exposure *(33, 34)*. These programmes may be targeted at all children of a certain age or only to children considered at risk. They specify the threshold blood lead concentration that indicates lead exposure is occurring (for example, 5 µg/dL) and the action that should be taken to identify the source and stop the exposure.

For the purposes of surveillance, laboratory-based methods with a high level of accuracy and precision and a low limit of detection are preferred. For screening purposes portable ASV instruments can be used, as they provide a low-cost and rapid method. However, results above the action threshold for the jurisdiction concerned should be confirmed by laboratory analysis.

7.4 Occupational health

The measurement of blood lead concentrations is often part of the routine monitoring of workers active in industries using lead. In many countries, the regular monitoring of blood lead concentrations of such workers is required by legislation, which also provides for the suspension or removal from further exposure of those with blood lead concentrations above certain values. The threshold value varies from country to country.

In this context, a method with a high level of accuracy and precision is required. The limit of detection required depends on the jurisdiction, though at the present time a method that is accurate down to 5 µg/dL is likely to be adequate.

8. Ethical considerations

When undertaking any kind of surveillance or exposure assessment study, it is important to adhere to national and international ethical principles and to the national legal framework as it applies to such an activity. A study should have the approval of the national ethical committee before sampling starts.

The study should be carried out in a way that upholds human rights, and respects, protects and is fair to the study participants and the community. In addition, the study should be scientifically sound and expected to yield useful information that can be acted upon if necessary. Some key requirements are listed below *(35)*.

- The purpose of the study should be legitimate, should be clearly defined and should be explicitly explained to all those involved, including the subjects from whom blood samples will be taken.
- Written, informed consent should be obtained from study subjects.
- Informed consent requires that the following information is provided:
    - the study or survey objective;
    - the targeted population and recruitment method;
    - possible risks and benefits to the participants;
    - approval of the study or survey protocol by an ethics committee;
    - the right to refuse consent or to withdraw consent at any time without giving reasons and without being subject to any form of discrimination;
    - the right to access personal results and for participants to decide whether they want to know or not to know their personal results;
    - the procedure for dealing with high blood lead concentrations;
    - the recipients of the study or survey data;
    - measures to ensure the confidentiality of personal data;
    - rights under national data protection regulations.

When individuals are told their results, they should also receive an explanation of the health significance of the result, whether further evaluation or an intervention is needed, and how to obtain this. Communication of the results to participants should be done professionally, using someone experienced in this communication. The potential need for further follow-up and a means for providing this should be considered at the planning stage of the study.

9. Conclusions

Lead exposure is common, particularly in countries where sources are poorly regulated. As lead exposure is harmful to health and can cause lifelong effects, it is important to identify exposed people and take the necessary preventive and mitigation measures as quickly as possible. Measurement of the blood lead concentration is the most widely accepted method for identifying lead exposure, and having the possibility to carry out this analysis is important for public health, occupational health and the clinical management of lead poisoning. Ideally, every country should have access to an accredited or proficiency-assured reference laboratory that can quantify lead in blood.

Point-of-care devices are available and have a role in screening for lead exposure. While they have been used to guide clinical management in extreme circumstances, this use should be validated by laboratory measurements.

Establishing and sustaining a laboratory service to perform blood lead analyses requires significant investment and resources; however, the same analytical equipment can be used to quantify other substances of interest, such as mercury. Thus, it may be possible to make a business case to set up a laboratory or to add the service to an existing laboratory. Alternatively, services can be sought from a suitable laboratory elsewhere (locally or abroad).

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Annex:
External quality assessment schemes for laboratories that analyse lead in blood

Table A1.1 provides information about a number of organizations that provide an external quality assessment service for the measurement of lead in blood. Some of these organizations provide an international service as well as a national one. The listing of a service does not imply endorsement by the World Health Organization.

Country/ region	Programme or organization	Website[5]
Canada	Quebec Multielement External Quality Assessment Scheme (QMEQAS)	https://www.inspq.qc.ca/en/ctq/eqas/qmeqas/description
Europe	European network of organizers of EQAS for occupational and environmental laboratory medicine	http://www.trace-elements.eu/default.aspx
Germany	German external quality assessment scheme for analyses in biological materials Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg	http://www.g-equas.de/
Spain	Programa Interlaboratorios de Control de Calidad de Plomo en Sangre [Interlaboratory Programme for Quality Control of Lead in Blood] Instituto Nacional de Seguridad e Higiene en el Trabajo [National Institute for Safety and Hygiene at Work]	https://www.insst.es/plomo-en-sangre.-picc-pbs
United Kingdom	UK National External Quality Assessment Service	http://www.ukneqas.org.uk
	Trace elements external quality assessment scheme, Surrey Pathology Services, Guildford	http://www.surreyeqas.org.uk/trace-elements-teqas/

5 Accessed on 10 July 2020.

Country/ region	Programme or organization	Website[5]
United States of America	Proficiency testing College of American Pathologists	http://www.cap.org/web/home/lab/proficiency-testing?_adf.ctrl-state=drowmm178_74&_afrLoop=366112906284229
	Toxicology proficiency testing Division of Chemistry and Toxicology, Bureau of Laboratories, Department of Health, Commonwealth of Pennsylvania	http://www.health.pa.gov/Your-Department-of-Health/Offices%20and%20Bureaus/Laboratories/Pages/Chemistry/Proficiency-Testing.aspx#.WIsJ2KmkpSE
	New York State biomonitoring proficiency testing programme for trace elements in whole blood, urine and serum Wadsworth Center, New York State Department of Health	https://www.wadsworth.org/programs/ehs/inorganic-analytical-chem/trace-elements
	Proficiency testing Toxicology Section, Wisconsin State Laboratory of Hygiene	http://www.slh.wisc.edu/proficiency/
	Lead and Multielement Proficiency Program (LAMP) Laboratory Quality Assurance and Standardization Programs United States Centres for Disease Control and Prevention	http://www.cdc.gov/labstandards/lamp.html

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