Module C.i.

Analytical Methods for Measuring Lead in Blood

LEAD PAINT ALLIANCE

GLOBAL ALLIANCE TO ELIMINATE LEAD PAINT

Outline

- Background
- Essentials of sample collection
- Brief information on different analytical methods
- Quality control considerations
- Summary
- References
- Disclaimer
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Background

- Lead exposure is primarily assessed through measurement in whole blood.
- The most common laboratory methods to measure blood lead concentrations are:
  - Anodic Stripping Voltammetry (ASV)
  - Atomic Absorption Spectrometry (AAS)
  - Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Analytical methods differ in their limit of detection, accuracy, costs and technical requirements (e.g. sample preparation, calibration, and skilled personnel)

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Sample collection
Care is needed

- Essential to avoid external contamination of the sample.
  - Personnel should be trained in good sampling and handling techniques to avoid contamination.
  - Collect, store and transport samples in a lead-free environment.
  - Thoroughly cleanse the skin around the puncture site.
  - Use lead-free sampling equipment and tubes. If not available send 'blanks' from same batch to the laboratory for testing of background lead content.
- Observe universal biosafety precautions.

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Sample collection
Care is needed

- Collect whole blood in a tube containing EDTA or heparin.
  - Invert the filled tube 8–10 times to ensure adequate mixing.
  - Clotted samples should be rejected – analytical results will be unreliable.
- Make sure to label the tube with the patient's identification details.
- Refrigerate samples (<4ºC) that are awaiting analysis – do not freeze.
  - Note: does not apply to samples measured using point-of-care device, which should be kept at room temperature.

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Choice of analytical method is determined by resources and needs

- Resource issues include:
  - availability of trained laboratory staff;
  - cost of reagents and other materials e.g. special gases, compressed air;
  - typical number of analyses needed (cost per analysis)
    - economy of scale possible with methods that allow multiple analyses;
  - special operating requirements e.g. reliable electricity supply, cooling water.

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Choice of analytical method is determined by resources and needs

- Required limit of detection varies according to the reason for the analysis.
- Population studies – may need a method accurate to <1 µg/dL
  - e.g. geometric mean blood lead concentration in USA in 2011–12 was 0.973 µg/dL.
- Confirmation of lead exposure and decisions on management – method accurate to 5 µg/dL acceptable.
  - Note: method may need to go to >65 µg/dL in severe cases of poisoning

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Anodic stripping voltammetry (ASV)

- Both laboratory-based and point-of-care devices available.
- EDTA is the preferred anticoagulant for laboratory method.
- Can analyse small samples: 50–100 µL.

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Anodic stripping voltammetry (ASV)
Laboratory method

- Relatively low-cost.
- Requires skilled laboratory technician and good quality reagents for best results.
- Sample pre-treatment is needed.
- Typical analytical range is 1–100 μg/dL, but greatest precision at blood lead concentrations >10 μg/dL.
- May be interference from elevated blood copper.
- Largely superseded by other methods.

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ASV: Point-of-care device
Considerations & limitations

- Portable device, can run on batteries – can be taken to the site.
- Uses a finger-prick (capillary sample), though venous samples can also be used.
- Equipment is supplied with calibration device and controls for high and low blood lead concentrations.

Point-of-care device

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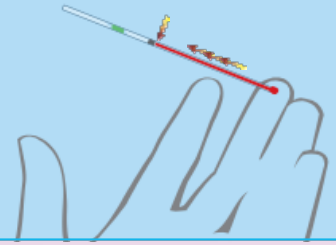
ASV: Point-of-care device
Considerations & limitations

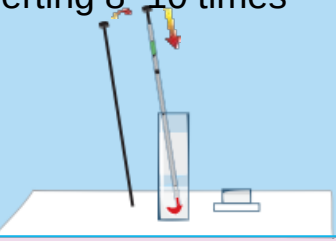
- Laboratory technician is not required to perform measurement – any scientifically competent person can be trained to use the equipment.
- Result available within minutes so immediate decisions can be made about management.

(Pictures adapted from reference 1)

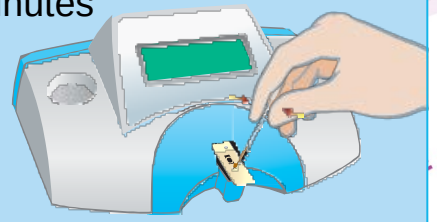
Collect capillary or venous sample



Put blood into a treatment reagent tube and mix by inverting 8–10 times



Place a drop of sample on sensor. Results in 3 minutes



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ASV: Point-of-care device
Considerations & limitations

- Widely-used device, LeadCare II, has analytical range of 3.3 - 65 µg/dL.
- Has comparable accuracy with laboratory-based methods.
- Elevated blood lead concentrations should, however, be confirmed with a laboratory-based method.
- Some experience of using LeadCare II to measure higher blood lead concentrations by diluting the sample. (Reference 2)

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ASV: Point-of-care device
Considerations & limitations

- Risk of sample contamination is high – extra care is needed:
  - finger-prick site likely to be highly contaminated with lead and needs thorough cleansing;
  - location of exposure likely to be highly contaminated e.g. with dust, so samples should be taken and analysed in a clean room.

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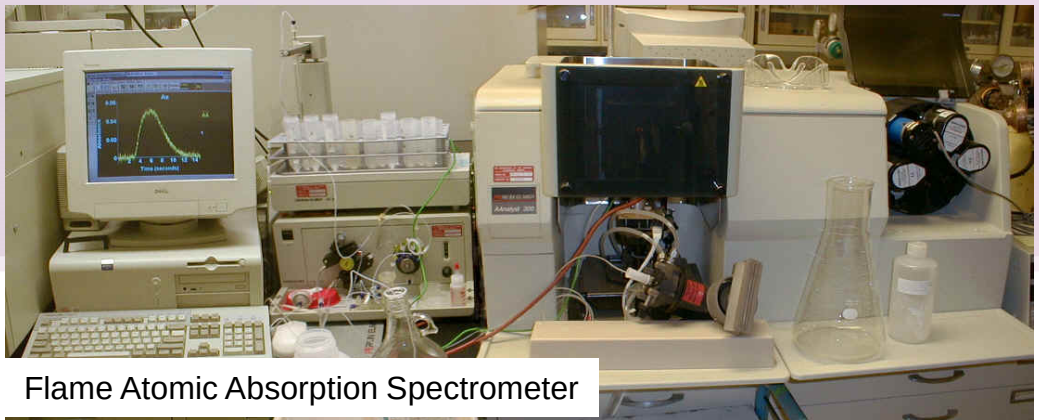
Atomic Absorption Spectrometry (AAS)

- Two techniques: Flame Atomic Absorption Spectrometry (FAAS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS).
- Methods differ in sample size needed, limits of detection and complexity of sample preparation.



Flame Atomic Absorption Spectrometer

Graphite Furnace Atomic Absorption Spectrometer

Flame Atomic Absorption Spectrometry (FAAS)

- Relatively easy to use and moderate cost.
- Needs special gases.
- Can be fitted with autosampler so multiple samples can be processed.
- Limit of detection depends on sample preparation and method used
  - at best: ~10 µg/dL with sample size of 50–100 µL.

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Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

- Requires skilled laboratory technician.
- Needs special gases.
- Can analyse very small samples: 10-50 μL.
- Methods available that can measure lead concentrations <0.1 μg/dL, though in routine use limit of detection is around 1–2 μg/dL.
- Can be fitted with autosampler so large number of samples can be run.
- Can be set up to measure multiple trace elements.

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Inductively-coupled plasma mass spectrometry (ICP-MS)

- Expensive and has high running costs
  - more economical if used for large sample runs.
- Requires highly-skilled laboratory technician.
- Very low limit of detection: 0.1 µg/dL.
- Can measure multiple elements from a small sample (50–100 µL).
- Can determine isotope ratio, which may help to identify the source of the lead.

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Lead isotope ratios can help to identify source

- Four main isotopes of lead are 208, 206, 207, 204.
- Ratios of the isotopes vary by source of the ore.
- Isotope ratio of soils represents mixing of lead from the various ores used in gasoline, consumer products and smelting.
- If isotope ratio in a lead source and in blood can be characterized, then this can be useful ‘fingerprinting’ of environmental pollution.

(Reference 3)

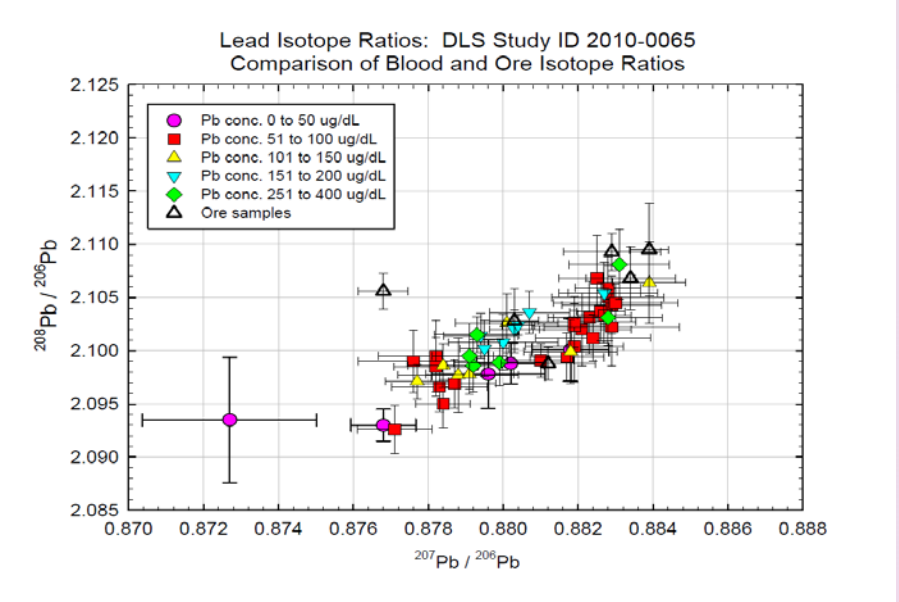

Chart shows group of children exposed to same source of lead and an individual exposed to a different source.
(Reference 4)

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Quality control considerations

- Important that analytical results are reliable.
- Laboratory should have in place an adequate quality management system e.g.:
  - standard operating procedures;
  - documented training and monitoring of staff performance;
  - use of certified reference standards;
  - internal quality control procedures – daily checks of analytical accuracy;
  - participation in external quality assessment scheme e.g. US LAMP.

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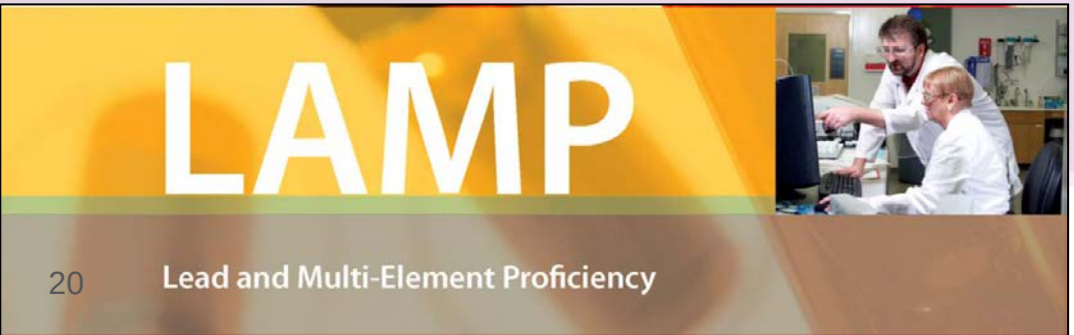
Laboratory quality assurance - LAMP

- A voluntary program that focuses on assuring the quality of blood lead, cadmium, and mercury analyses.
- Each quarter US CDC provides spiked blood samples, which are analyzed by participating laboratories who return the results to CDC.
- CDC provides detailed reports on the laboratories about how well they performed these analyses.
- No charge for participation.



Centers for Disease Control and Prevention (CDC)
Lead and Multi-Element Proficiency
4770 Buford Highway N.E., Mailstop F-18
Atlanta, GA 30341-3724 USA

Fax number: (770) 488-4097
E-mail address: LAMP@cdc.gov

Summary

- Whole blood is the preferred sample for assessing exposure to lead.
- Adequate measures should be taken to avoid sample contamination.
- A range of analytical methods are available – the decision about which one to use is determined by the available resources and the limit of detection required.
- Quality assurance procedures are important to ensure the reliability of analytical results.

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3. Komárek M et al. Lead isotopes in environmental sciences: A review. Environment International. 2008; 34:562–577

4. Brown MJB, US Centers for Disease Control and Prevention, personal communication, 2015

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Additional references

Sample collection

Step-by-step guide for collecting capillary sample. US Centers for Disease Control and Prevention.

Poster: http://www.cdc.gov/labstandards/pdf/vitaleqa/Poster_CapillaryBlood.pdf

Video demonstration: http://www.cdc.gov/nceh/lead/training/blood_lead_samples.htm

Guidelines on drawing blood: best practices in phlebotomy. Geneva: World Health Organization; 2010 (http://www.who.int/injection_safety/sign/drawing_blood_best/en/, accessed 3 January 2017).

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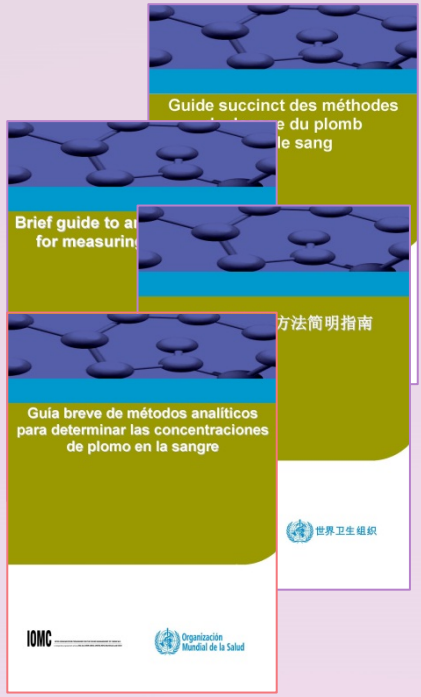
Analysis

Brief guide to analytical methods for measuring lead in blood. Geneva: World Health Organization; 2011 (available in Chinese, English, French and Spanish) (http://www.who.int/ipcs/assessment/public_health/lead/en/, accessed 9 February 2017)



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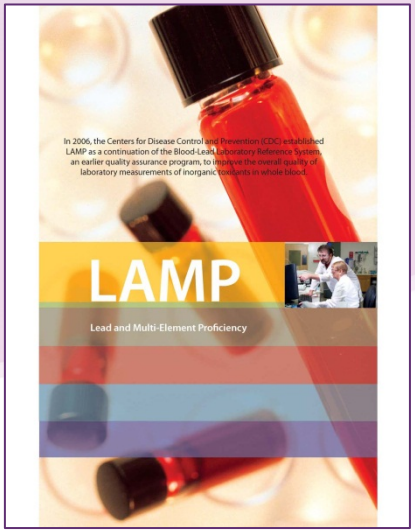

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