# Analytical Methods for Measuring Lead in Blood

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# Are Elevated Blood Lead Levels Still A Problem?

| Country | Author/Year | Mean BLL µg/dl | % greater than 10 |
|---|---|---|---|
| India | Kalra et al., 2013 | 5.3 | 12 |
| China | Xie et al., 2013 | 4.3 | 4.78 |
| South Africa | Naicker et al., 2013 | 7.9 | 25 |
| Democratic Republic of Congo | Tuakuila et al., 2013 | 11.5 | 71 |
| Thailand | Swaddiwudhipong et al., 2013 | 9.8 | 43.3 |
| Saudi Arabia | El-Desoky et al., 2014 | 5.2 | 17.8 |
| Nigeria | Ugwuja et al., 2014 | 8·7 | 33 |
| China | Hou et al., 2013 | 8.8 | NA |
| Bangladesh | Gleason et. al. 2014 | 8 | 26 |
| | **Mean** | **7.6** | **29.1** |
| **USA** | **NHANES 2010/ GM 1.3 ug/dl** | **NA** | **0.8** |

# Module C.i.

## Analytical Methods for Measuring Lead in Blood


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GLOBAL ALLIANCE TO ELIMINATE LEAD PAINT

# Outline

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

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# Background

- Assessment of lead exposure is primarily performed using 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
- See also references C.i.2 and C.i.3

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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
  - NB 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 some methods
  - 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 and accuracy vary according to the reason for the analysis
- Population studies – may need a method accurate to 1-2 µg/dL
  - e.g. geometric mean blood lead concentration in USA is 1.3 µg/dL
- Confirmation of lead exposure and decisions on management – method accurate to 5 µg/dL acceptable
  - NB method may need to go to >65 µg/dL in severe cases of poisoning


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# Examples of analytical equipment

Graphite Furnace Atomic Absorption Spectrophotometer

Inductively Coupled Plasma Mass Spectrometer


LeadCare I


LeadCare II

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# Anodic stripping voltammetry (ASV)

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

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# Anodic stripping voltammetry (ASV)
## Laboratory

- Relatively low-cost
- Requires skilled laboratory technician and good quality reagents for best results
- Sample pre-treatment 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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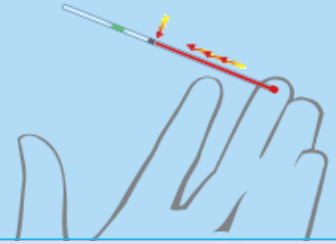
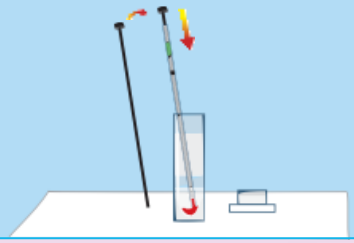
# ASV Point-of-care device
## Considerations & limitations

- Portable device, can run on batteries – can be taken to the site
- Risk of sample contamination is high:
  - Finger-prick site likely to be highly contaminated and needs thorough cleansing
  - Site of exposure likely to be highly contaminated e.g. with dust, so samples should be taken and analysed in a clean room
- Only one brand – LeadCare – must use reagents supplied with the equipment

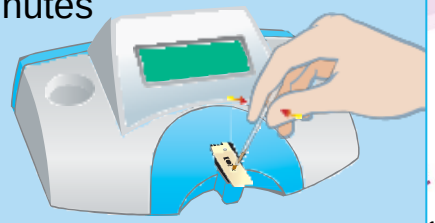
Collect capillary or venous sample



Put blood into a treatment reagent tube and mix



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



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# ASV point-of-care device
## Considerations & limitations

- LeadCare II analytical range is 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 C.i.1.

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# ASV point-of-care device
## Advantages

- 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
- Equipment is supplied with calibration device and controls for high and low blood lead concentrations

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# Atomic Absorption Spectrometry (AAS)

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

# 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

# Lead isotope ratios

- 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 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 C.i.2

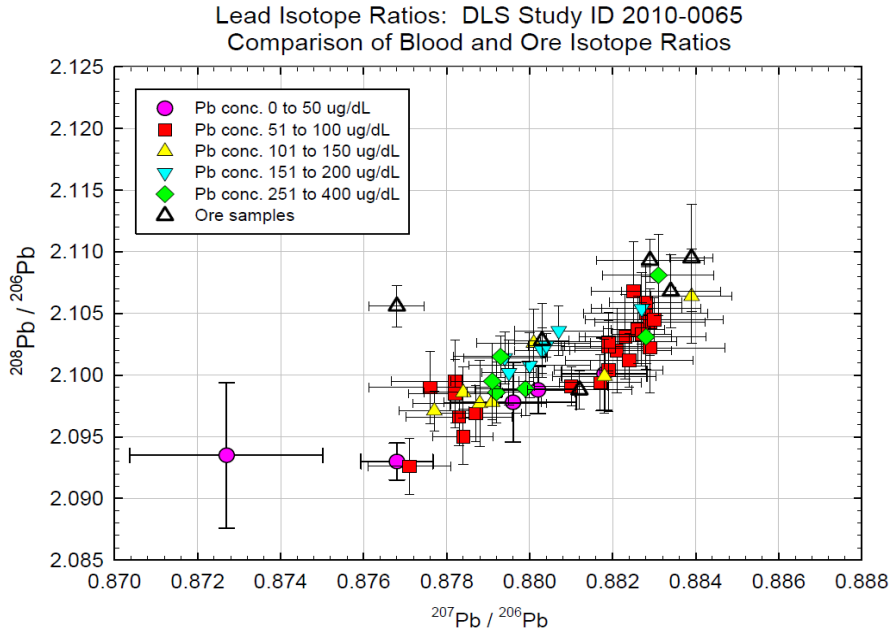


Chart shows group of children exposed to same source of lead and an individual exposed to a different source
Reference C.i.3

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# Quality control considerations

- Important that analytical results are reliable
- Laboratory should have in place adequate quality assurance measures 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 control programmes  e.g. US LAMP

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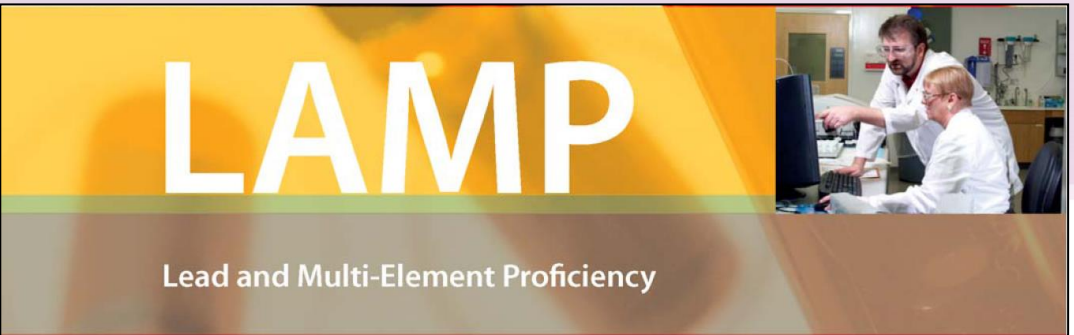
# Laboratory quality assurance - LAMP

- A voluntary program that focuses on assuring the quality of blood lead, cadmium, and mercury levels
- Each quarter US CDC provides 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

# References

Based upon presentations made at the Global Alliance to Eliminate Lead Paint Workshop on Establishing Legal Limits on Lead in Paint, 22 – 23 September 2014, New Delhi, India. Adapted for inclusion in the Lead Paint Alliance "Toolkit" for Governments, April 2015


C.i.1. Neri AJ et al. (2014) Analysis of a novel field dilution method for testing samples that exceed the analytic range of point-of-care blood lead analyzers. Int J Environ Health Res; 24(5):418-428)

C.i.2. Komárek M et al (2008). Lead isotopes in environmental sciences: A review. Environment International 34 (2008) 562–577

C.i.3. Brown MJB (2015), US Centers for Disease Control, personal communication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# References - general

**Sample collection**

C.i.4.Step-by-step guide (CDC)
http://www.cdc.gov/labstandards/pdf/vitaleqa/Poster_CapillaryBlood.pdf

C.i.5. Video demonstration (CDC)
http://www.cdc.gov/nceh/lead/training/blood_lead_samples.htm


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# References - general

**Analysis**

C.i.6. Brief guide to analytical methods for measuring lead in blood (available in Chinese, French, English and Spanish)
http://www.who.int/ipcs/assessment/public_health/lead/en/

C.i.7. CDC Lead and Multi-element Proficiency programme (LAMP)
http://www.cdc.gov/labstandards/lamp.html


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