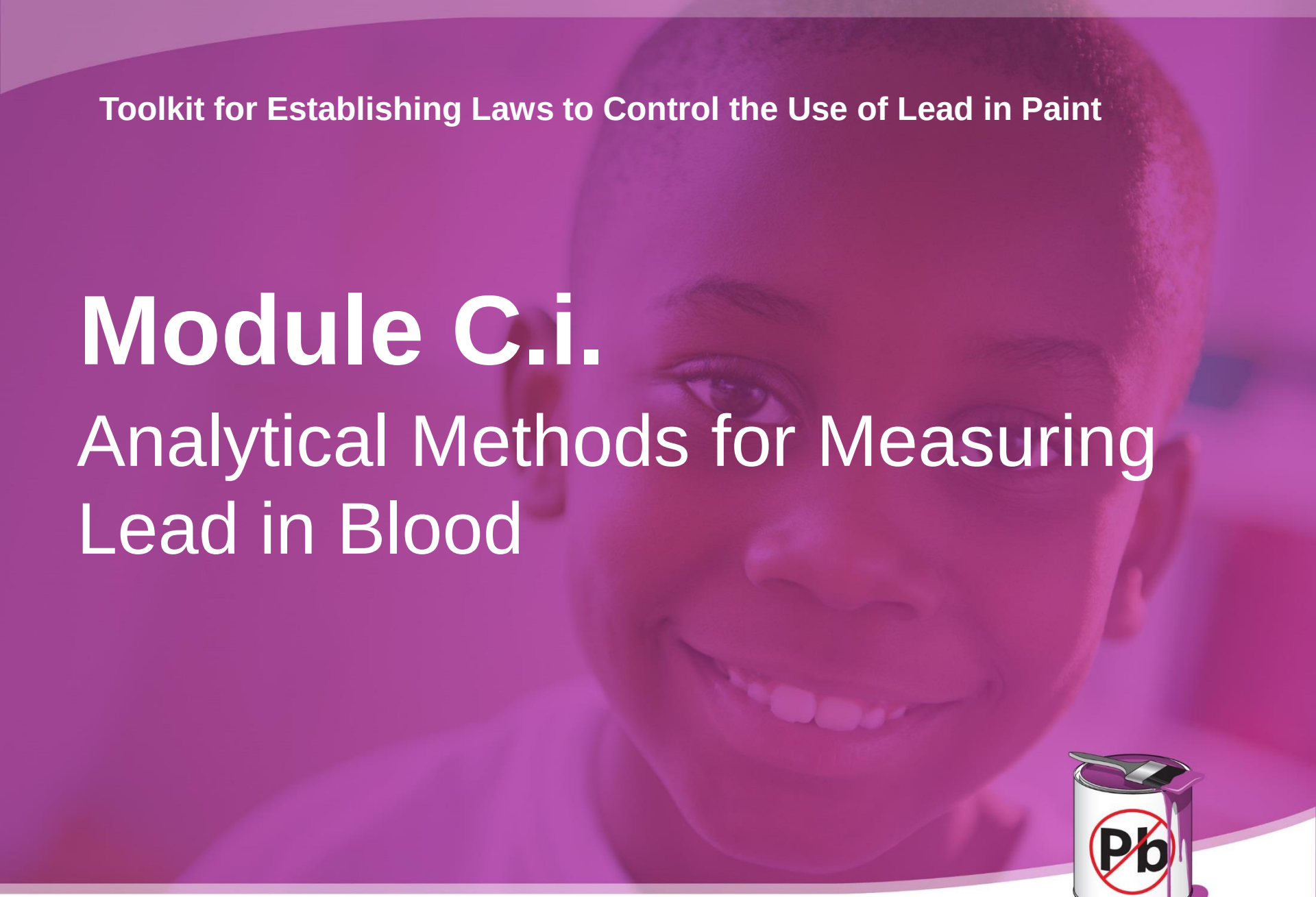
Module C.i.

Analytical Methods for Measuring Lead in Blood



GLOBAL ALLIANCE TO ELIMINATE LEAD PAINT

LEAD PAINT ALLIANCE

Outline

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

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Why and how to assess human exposure to lead?

WHY:

- To evaluate human exposure to lead from all potential sources;
- To assess a risk to human health;
- To prognoses the burden of ill-health and disease;
- To provide scientific bases for decision-makers, policy and legislation development;
- To identify prevail exposure source and recommend on effective ways on exposure prevention;
- To demonstrate effectiveness of preventive measures.

HOW:

- Air, drinking water, food and consumer products monitoring;
- Human biomonitoring.

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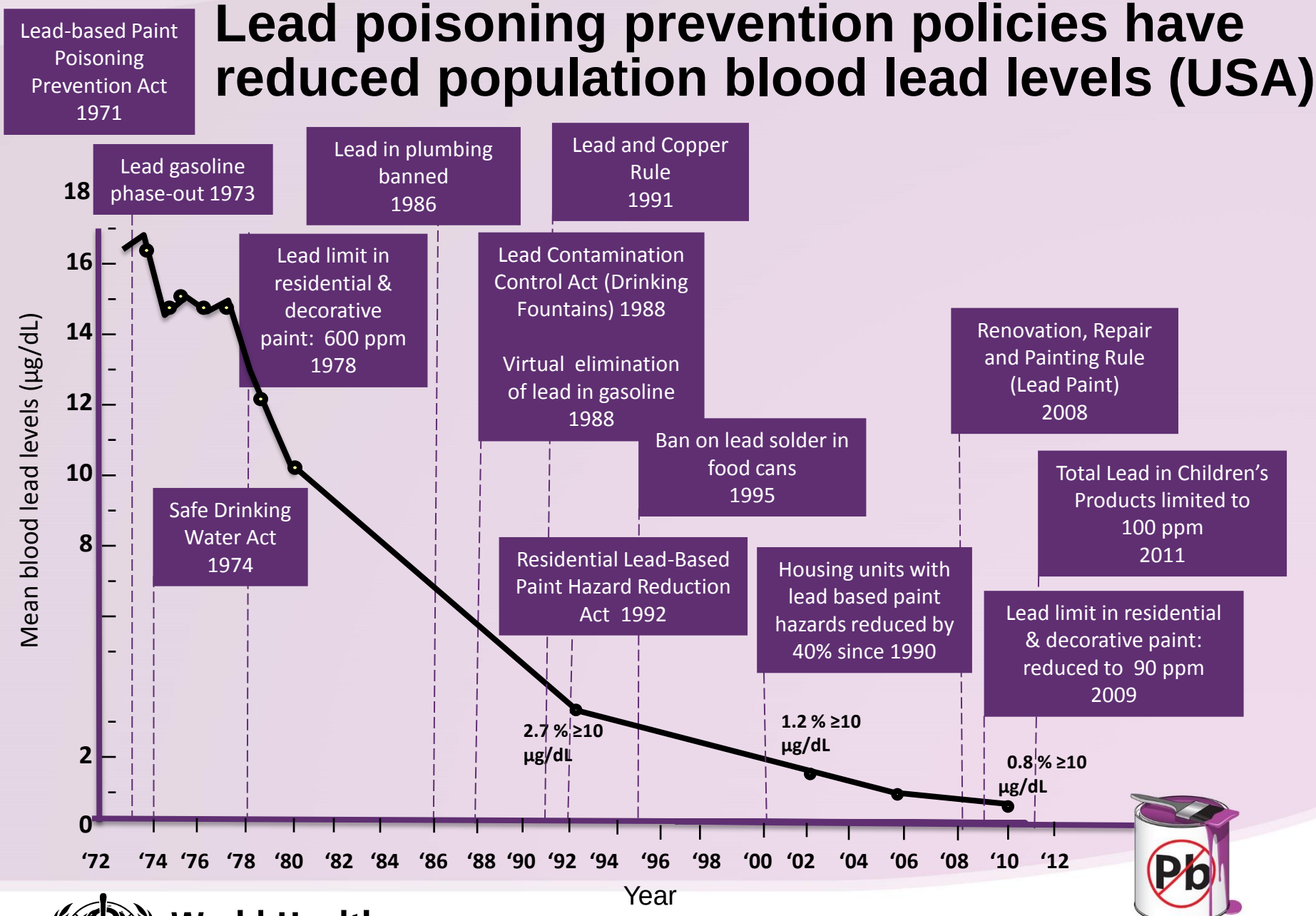
Lead poisoning prevention policies have reduced population blood lead levels (USA)



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HBM: why do we need a human biomonitoring

- PROs

HBM provides the most direct metric for human risk integrating the impacts of all

- sources and pathways
- geographic locations and microenvironments,
- activities and consumer products
- exposure media and routes of entry

- CONs

- Can NOT replace environmental and consumer products monitoring; they are complimentary;
- Not always applicable due to cultural, technical and other reasons
- Planned and performed inappropriately can lead to incorrect conclusions

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Background

- Main steps: blood sampling – samples transportation – samples analysis
- 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)

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Sample collection

- 2 main principles

  don’t harm patient!

  don’t contaminate samples

- 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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Pre-analysis samples treatment

- Prevention of blood coagulation - 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
- 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

NB! Make sure to label the tube with the patient's identification details

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Choice of analytical method: criteria

- Investigation purpose
- 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

- Availability of trained laboratory staff
- **Note: method may need to go to >65 µg/dL in severe cases of poisoning**
- 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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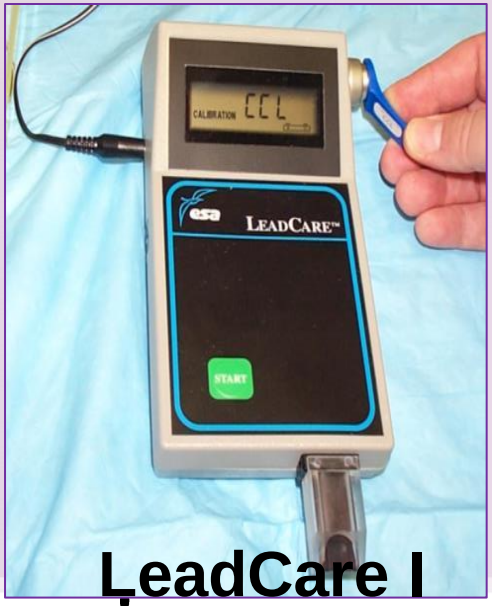
Examples of analytical equipment

Graphite Furnace Atomic Absorption Spectrophotometry

Inductively Coupled Plasma Mass Spectrometry

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



LeadCare I

LeadCare II

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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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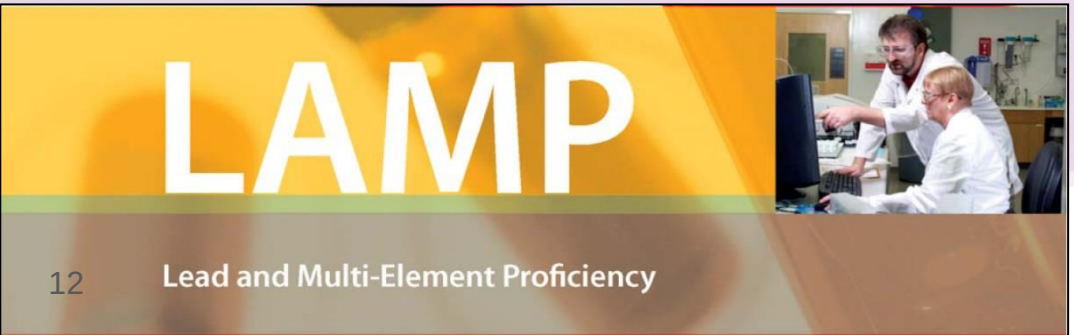
Laboratory quality assurance - LAMP

- 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

Anodic stripping voltammetry (ASV)
Laboratory method

- Both laboratory-based and point-of-care devices available
- Relatively low-cost
- Requires skilled laboratory technician and good quality reagents for best results
- Sample pre-treatment is needed (EDTA is the preferred anticoagulant)
- 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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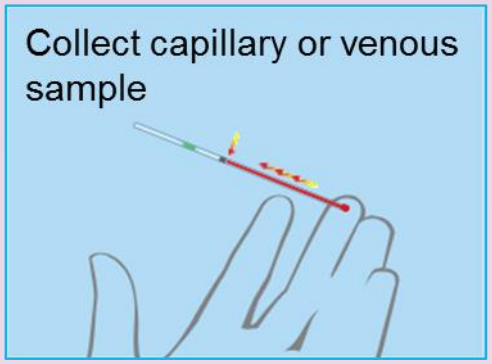
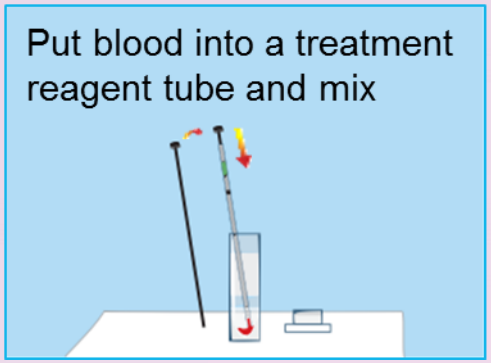
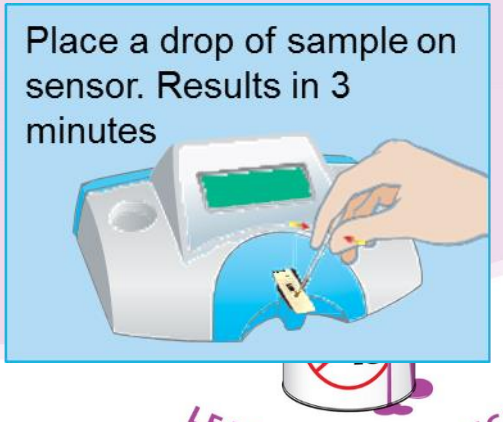
ASV: Point-of-care device Considerations & limitations

- Portable device, can run on batteries – can be taken to the site
- Uses a finger-prick (capillary sample)
- Equipment is supplied with calibration device and controls for high and low blood lead concentrations
- Result available within minutes and immediate decisions can be made about management







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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 1)

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ASV: Point-of-care device Considerations & limitations

- Risk of sample contamination is high – extra care needed
  - 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 – so must use reagents supplied with the equipment

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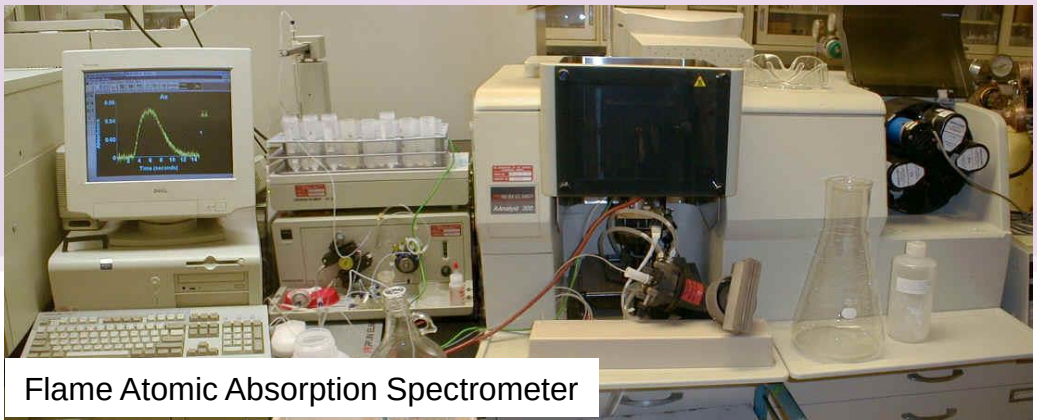
Atomic Absorption Spectrometry (AAS)

- 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

- 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 2)

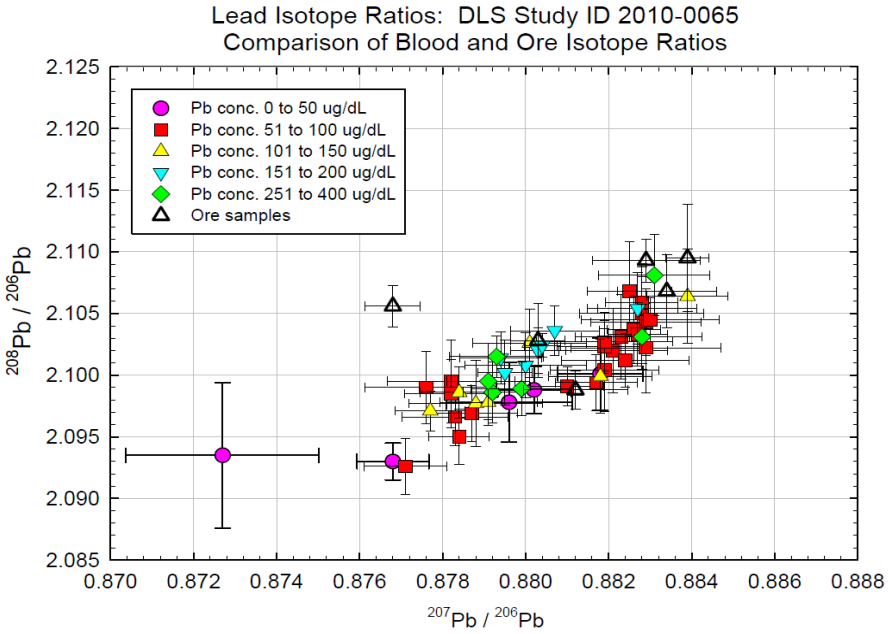

Chart shows group of children exposed to same source of lead and an individual exposed to a different source (Reference 3)

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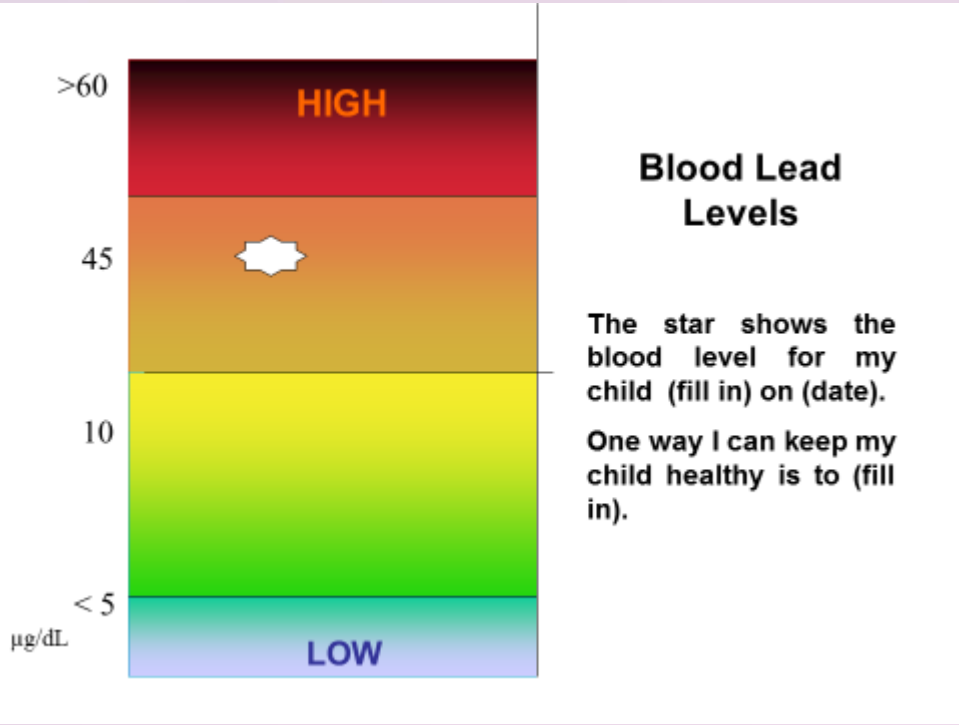
Reporting results (cont.)



- A form such as this one can be used to graphically explain blood test results. The "star" is moveable so the child's actual result can be displayed. The form can be modified for use with environmental sample results.

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Regional experience

- Majority of countries in the Region have some experience in lead HBM

  - Russian Federation in the frame of WHO Europe initiative to monitor Parma Declaration commitments implementation

  - Kazakhstan – a number of contaminated sites

  - Belarus – occupation safety studies

  - Serbia – contaminated sites

  - ….

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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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References

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)

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

3. Brown MJB (2015), US Centers for Disease Control and Prevention, personal communication

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Additional references

Sample collection

CDC. Step-by-step guide for collecting capillary sample
http://www.cdc.gov/labstandards/pdf/vitaleqa/Poster_CapillaryBlood.pdf

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

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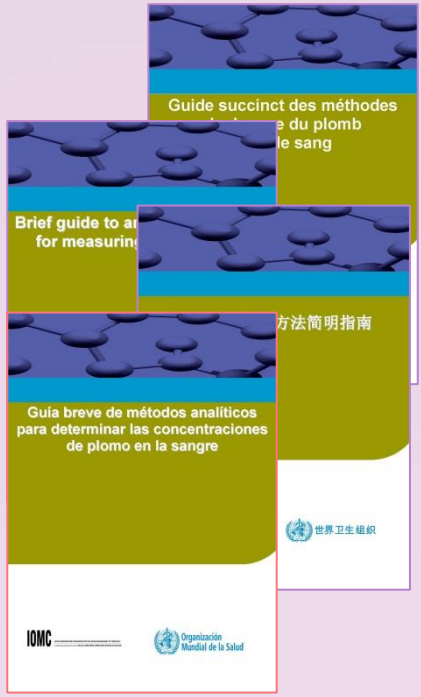
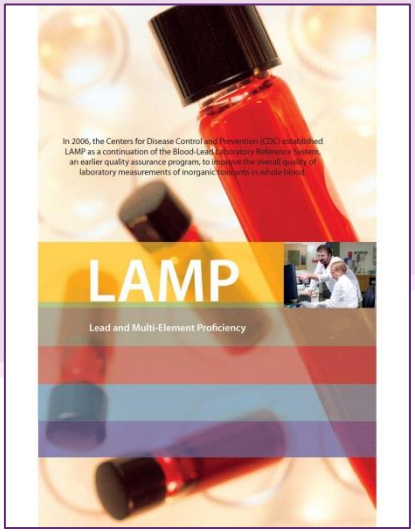
Additional references

Analysis

WHO (2011). Brief guide to analytical methods for measuring lead in blood (available in Chinese, English, French and Spanish)
http://www.who.int/ipcs/assessment/public_health/lead/en/



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



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