

Uncertainty of Measurement of the Analysis of Lead in Blood by Graphite Furnace AAS
Calibrating with a Commercial Available Standard
Version 1

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Analysis Principle

Whole blood samples are diluted with a modifier and then injected directly into the graphite furnace of the atomic absorption spectrophotometer.

The Calibration Process

Standards

Stock Lead Solution 4.8mmol/L

1. Commercially available 1000ppm Pb(NO₃)₂ standard equivalent to 4.826mmol/L

Working Lead Solution 4.8µmol/L

1. Dilute 1.0 mL of Stock Lead Solution to 1000 mL with distilled water. This solution should be prepared daily.

Standard preparation

1. Prepare calibration solutions by adding 0, 0.1,0.2,0.3,0.4, and 0.5mLs of Working Lead Solution (4.8µmol/L) to separate polypropylene centrifuge tubes.
2. To each tube add 1.00mL of porcine or human blood containing less than 0.5µmol/L lead.
3. Add matrix modifier/diluent solution (0.04M Diammonium hydrogen phosphate + 0.05% Triton X-100) to relevant tubes to give a final volume of 10mLs.
4. These solutions now correspond to 0, 0.06, 0.12, 0.18, and 0.24, 0.30 µmol Pb/L.

Sample Preparation

1. To sample tubes add 0.5mL of thoroughly mixed whole blood.
2. Add 4.5mL of modifier/diluent solution.
3. Cap and mix all tubes on rotator for five minutes.
4. Transfer about 1 mL of standards, controls and samples to individual sample cups on the auto sampler carousel of the GFAAS. From this 15 μ L aliquots will be injected into the graphite furnace of the GFAAS in duplicate.
5. On carousel position 40 place a cupful of modifier/diluent to be used as a sample blank.

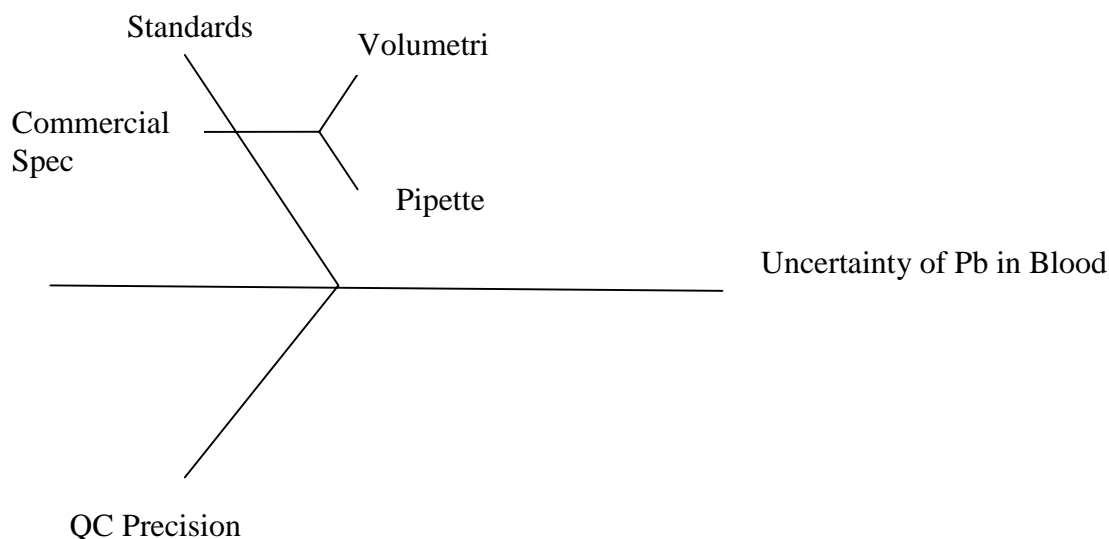


Figure 1. An Ishikawa (cause and effect) diagram of the uncertainty budget

Assumptions

1. The blood sample is considered homogeneous and any uncertainty due to deviation from homogeneity can be considered small in comparison to other uncertainties calculated
2. The chemistry of blood is relatively similar between samples. It has a very narrow pH range, and the amounts of different elements and compounds in it vary by small amounts. It certainly does not compare to environmental samples such as soil or water where elements or compounds can vary by orders of magnitude. Lead in blood by GFAAS is a well established technique and much method validation and research has been done with this test. A textbook “Graphite Furnace AAS – A Source Book” by Walter Slavin⁸ has extensively covered this determination and has made no references to differences in individuals blood requiring any extra analysis preparation. As the standards are made up in the

blood matrix, albeit porcine blood, different blood batches had been used over the time period that the standard curve had been calculated. Hence, this error is somewhat included in that estimate.

3. The QC data was calculated using a Biorad QC Blood Lead Seronorm Level 2 Batch # 404108 and was analysed 61 times from April '98 to May '00 with an average of 1.94 μ mol/L.
4. The uncertainty of the standard curve was not included in the uncertainty determination because the variation of the absorbance within days and between days is included in the estimate of the uncertainty of the QC sample.

Calculation of Uncertainty of Lead in Blood

Uncertainty of Stock Standard Solution

The commercially available Pb Standard solution 1000ppm (APS Ajax Finechem, Sydney, Australia) has a manufacturer's stated uncertainty of $1000 \pm 5\text{mg/L}$. The value of the uncertainty is given without a confidence level or distribution information, so an assumption is necessary. Without any other information a rectangular distribution is assumed and therefore the semirange is divided by $\sqrt{3}$. This uncertainty is then converted to the units mmol/L as they are the units that the analysis is performed in.

$$\begin{aligned}U_{\text{Stock}} &= \frac{5}{\sqrt{3}} \\ &= 2.887\text{mg} \\ \text{Convert to mmol/L} &= \frac{\text{mg}}{\text{Mol Wt}} \\ &= \frac{2.887}{207.2} \\ &= 1.393 \times 10^{-2} \text{mmol/L}\end{aligned}$$

The uncertainty of the pipetter used was calculated from the compilation of the six-monthly calibrations of all the pipettors of that range used in the laboratory. The calibrations were performed by different analysts in the laboratory over a 5 year period. From this data a relative standard deviation (RSD) was calculated for the use of that type of pipetter.

Pipetter A (100 μL – 1000 μL) using 1mL

The manufacturer did not specify a confidence level with the pipetter tolerance, therefore, a rectangular distribution is assumed and the specification is divided by $\sqrt{3}$.

These two contributions are combined to become the standard uncertainty of the pipetter.

$$\begin{aligned}
 \text{RSD} &= 0.804\% \\
 U_{\text{PipRepeat}} \text{ at } 1\text{mL} &= 0.00804\text{mL} \\
 \text{Manufacturer's Specification} &= \pm 0.7\% \\
 &\equiv \pm 0.007\text{mL} \\
 U_{\text{PipSpec}} &= \frac{0.007}{\sqrt{3}} \\
 &= 4.041 \times 10^{-3} \text{ mL} \\
 \\
 U_{\text{Pipetter}} &= \sqrt{(U_{\text{PipRepeat}})^2 + (U_{\text{PipSpec}})^2} \\
 &= \sqrt{(0.00804)^2 + (0.004041)^2} \\
 &= 8.998 \times 10^{-3} \text{ mL}
 \end{aligned}$$

The uncertainty of the 1L volumetric flask can be calculated by combining the three major influences of the volume, the calibration, the repeatability and the temperature effects.

(i) Calibration

The tolerance of a 1 Litre A Grade volumetric flask is given by the manufacture as $1000 \pm 0.4\text{mL}$ measured at a temperature of 20°C . There is no information given about the confidence level so this time an assumption is made that a triangular distribution is a good estimate of the distribution as volumetric glassware is considered reasonably accurate and in compliance with the manufacturer's specification.

$$\begin{aligned}
 \text{Tolerance} &= \pm 0.40\text{mL} \\
 U_{\text{Tol}} &= \frac{0.40}{\sqrt{6}} \\
 &= 0.163\text{mL}
 \end{aligned}$$

(ii) The difference between the laboratory temperature and the calibration temperature

The manufacturer had calibrated the volumetric flask at a temperature of 20°C , however, the laboratory temperature varies between the limits of $\pm 3^\circ\text{C}$. The uncertainty from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The coefficient of volume expansion for water is $1.806 \times 10^{-4} \text{mL } ^\circ\text{C}^{-1}$ (calculated from data in the CRC "Handbook of Chemistry and Physics 74th Ed"⁹) which gives a volume variation of a 1 litre volumetric flask as calculated below

$$\pm V \times \text{Temp} \times \text{Coefficient of Expansion H}_2\text{O}$$

$$\pm 1000 \times 3 \times 1.806 \times 10^{-4}$$

$$= 0.542 \text{ mL}$$

Convert to standard uncertainty and assume rectangular distribution

$$U_{\text{Temp}} = \frac{0.542}{\sqrt{3}}$$

$$= 0.313 \text{ mL}$$

(iii) Repeatability

The random variation in filling up the volumetric flask was estimated by performing a repeatability experiment. The SD of 12 repeat weighings of a 1 Litre volumetric flask filled to the mark with H₂O was 0.128 mL and can be used as the standard uncertainty directly.

$$U_{\text{Repeat}} = 0.128 \text{ mL}$$

The combined uncertainty of the 1L volumetric flask was calculated using Rule 1 (square root of the squares) of the effects.

$$\begin{aligned} U_{\text{volumetric}} &= \sqrt{(U_{\text{Tol}})^2 + (U_{\text{Temp}})^2 + (U_{\text{Repeat}})^2} \\ &= \sqrt{(0.163)^2 + (0.313)^2 + (0.128)^2} \\ &= 3.754 \times 10^{-1} \text{ mL} \end{aligned}$$

The combined uncertainty of standards can be calculated using Rule 2 as the relationship between the standard uncertainties is a quotient that is expressed by the units $\mu\text{mol/L}$

$$U_{\text{Stock}} = 1.393 \times 10^{-2} \text{ mmol/L}$$

$$U_{\text{Pipetter}} = 8.998 \times 10^{-3} \text{ mL}$$

$$U_{\text{Volumetric}} = 3.754 \times 10^{-1} \text{ mL}$$

$$\begin{aligned} U_{\text{Std}} &= 4.826 \mu\text{mol/L} \sqrt{\left(\frac{U_{\text{Stock}}}{\text{Conc}}\right)^2 + \left(\frac{U_{\text{Pipetter}}}{\text{Pipette}}\right)^2 + \left(\frac{U_{\text{Volumetric}}}{\text{Vol}}\right)^2} \\ &= 4.826 \times \sqrt{\left(\frac{1.393 \times 10^{-2}}{4.826}\right)^2 + \left(\frac{8.998 \times 10^{-3}}{1}\right)^2 + \left(\frac{3.754 \times 10^{-1}}{1000}\right)^2} \\ &= 4.826 \times 9.458 \times 10^{-3} \\ &= 4.564 \times 10^{-2} \mu\text{mol/L} \end{aligned}$$

Hence, the overall expanded uncertainty can be calculated by combining an uncertainty of the precision of the method with the combined uncertainty of the standards. The precision of the method was determined by calculating the SD of a commercially available quality control material that had been analysed by different analysts, in the same laboratory, on the same equipment over a 3 year period.

$$U_{\text{QC}} = 1.608 \times 10^{-1} \mu\text{mol/L} \text{ at } 1.94 \mu\text{mol/L}$$

$$U_{\text{Std}} = 4.564 \times 10^{-2} \mu\text{mol/L} \text{ at } 4.826 \mu\text{mol/L}$$

x = analytical result in $\mu\text{mol/L}$

$$\begin{aligned} U_{\text{Expanded}} &= 2 \times x \sqrt{\left(\frac{U_{\text{QC}}}{\text{QC}}\right)^2 + \left(\frac{U_{\text{Std}}}{\text{Std}}\right)^2} \\ &= 2 \times x \sqrt{\left(\frac{1.608 \times 10^{-1}}{1.94}\right)^2 + \left(\frac{4.564 \times 10^{-2}}{4.826}\right)^2} \\ &= 2 \times x \sqrt{(0.0828)^2 + (0.00945)^2} \\ &= 1.668 \times 10^{-1} \times x \end{aligned}$$

This gives a factor that can be included in a LIMS computer system to enable automatic calculation of the uncertainty from an entered result. The uncertainty contributions to the expanded uncertainty is shown in Figure 2 and the graph of the uncertainty over the concentration range can be seen in Figure 3.

$$\boxed{= 1.668 \times 10^{-1} \times x}$$

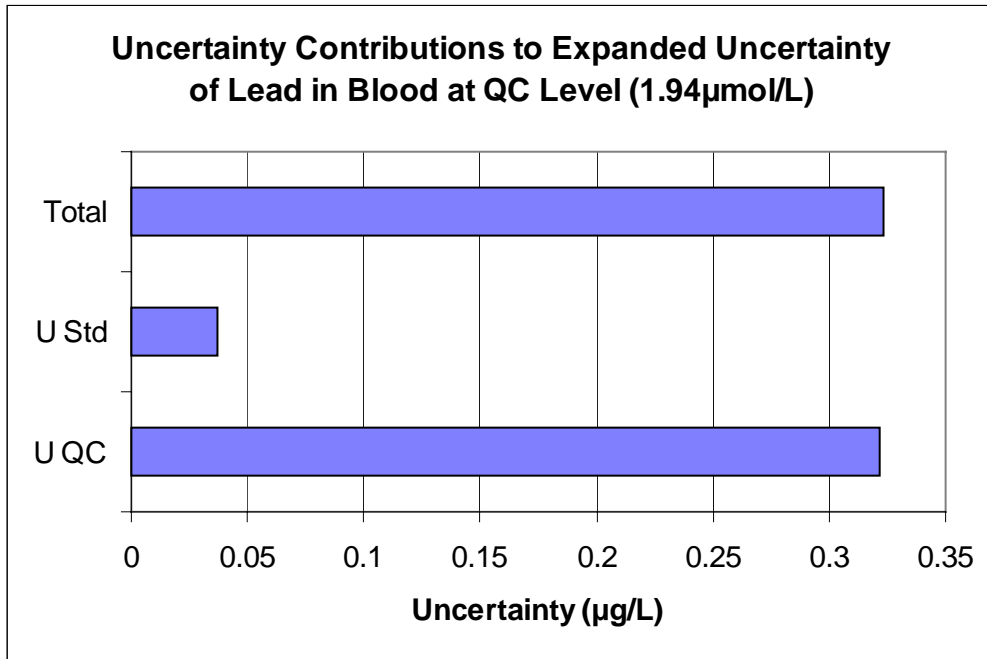


Figure 2. The uncertainty contributions to the expanded uncertainty of lead in blood at a concentration level of 1.94µmol/L

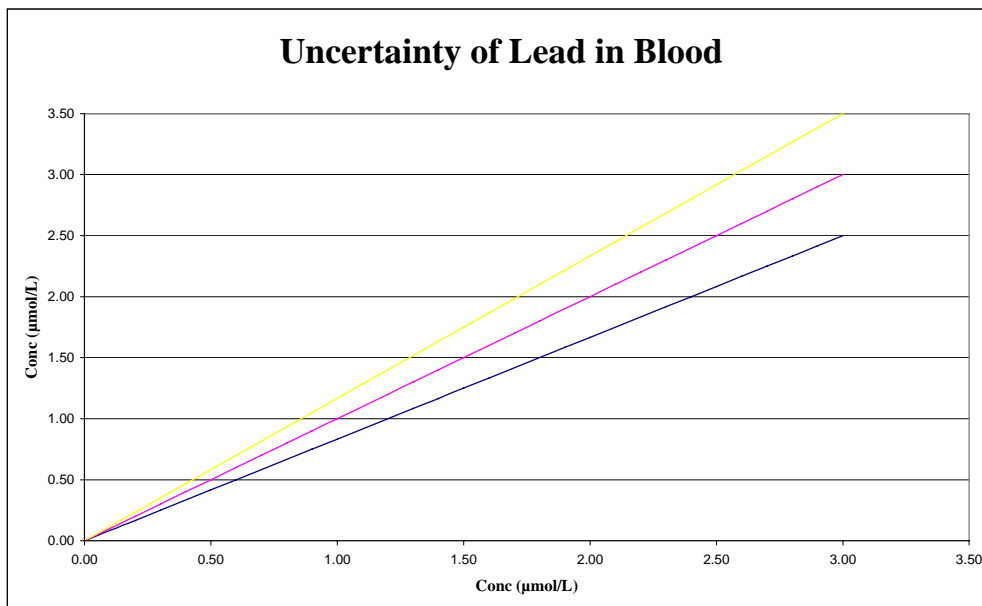


Figure 3. The uncertainty of lead in blood over the concentration range of 0.1 to 3.0µmol/L

References

1. Slavin W. Graphite Furnace AAS – A Source Book p109-120 Published by The Perkin-Elmer Corporation (1984)
2. Lide D. R. CRC Handbook of Chemistry and Physics 74rd Ed (1993 – 1994)