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Validation of determination of lead (Pb) in blood by electrothermal atomic absorption spectrometry (ETAAS) on the basis of interlaboratory comparison data

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Abstract

ISO 15189 standard establishes a requirement to periodically revalidate analytical methods for the determination of trace elements like Pb in blood, as conditions change and technical advances are made. The aim of this study was to revalidate an electrothermal atomic absorption spectrometry (ETAAS) method for determination of Pb in blood over the microrange 25–35 μg/dL, on the basis of historical results of interlaboratory comparison programmes. Precision and inaccuracy were estimated by analysis of records of an external quality control programme for Pb (PICC-PbS). The precision and inaccuracy values obtained were both less than 5%, highly satisfactory in view of the validation requirement that precision and inaccuracy be less than 10%. These findings demonstrate the effectiveness of this new validation methodology, which does not require any disruption of the laboratory's routine activity, and which can be used even if the method in question has not been validated previously at that laboratory.

Keywords: Lead; Accreditation; Validation; Intercomparison programmes; ISO 15189

Introduction

In laboratories dedicated to the determination of trace elements, the validation of analytical methods is a requisite for achieving ISO 15189 accreditation [1]. ISO 15189 standard states that "The laboratory shall use only validated procedures for confirming that the examination procedures are suitable for the intended use," that "The validations shall be as extensive as are necessary to meet the needs in the given application or field of application," and that "Procedures need to be periodically revalidated in view of changing conditions and technical advances" [1,2].

Inaccuracy and precision are two of the key validation parameters for any instrumental method for determination of a trace element. These parameters can be estimated on the basis of interlaboratory comparison programmes, or the external quality control programmes in which participation is required for ISO 15189 accreditation [1,3,4]. The present study aimed to estimate the inaccuracy and precision of an electrothermal atomic absorption spectrometry (ETAAS) method for determination of Pb in blood, within a

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narrow microrange (25–35 μg/dL), on the basis of historical results of participation in intercomparison programmes.

Material and methods

Inaccuracy and precision were estimated for method based on the determination of lead in whole blood. The concentration of Pb in whole blood, in μg/dL, was determinated by ETAAS with Zeeman correction and graphite furnace (THGA, graphite tubes with end caps) [5–8] in a Perkin-Elmer 4110-ZL apparatus. Analytical and instrumental parameters were: LCH lamp for lead, wavelength 283.3 nm, current 10 mA, slit width 0.7 nm, HNO_3 0.2% (diluyent), precision <5% and inaccuracy <5%.

All records of participation in the Lead Quality Control Interlaboratory Programme of the Aragonese Institute for Occupational Health and Safety (Zaragoza Office) (PICC-PbS) over the period 2000–2006 were analysed. From these data a series of paired values were obtained: V_L (Pb concentration in blood as given by the laboratory) and V_R (target value of the intercomparison programme). It was necessary to obtain at least 10 pairs of values, obtained by the same analytical method (ETAAS with Zeeman correction) within the range considered (25–35 μg/dL). For this methodology to be effective, it is advisable that the target value V_R be based on determinations in at least ten laboratories [3].

The steps followed for estimation of precision and accuracy (expressed as inaccuracy) are as follows:

(1) Determination of the difference (d) between each pair of values:

$$d = V_R - V_L.$$

(2) Calculation of the relative difference (accuracy, A) in each case:

$$A = [(V_R - V_L)/V_R] \times 100.$$

(3) Calculation of the mean difference and standard deviation of the differences (S_d), and the mean accuracy and standard deviation of the accuracies (S_A).
(4) Confirmation that means V_L does not differ significantly from mean V_R, using Student's t-test. Specifically, it is necessary that the calculated t value [(mean difference)/(S_d/(root n))] be less than the tabulated t-value.
(5) Calculation of accuracy, expressed as percentage inaccuracy, i.e. 100 minus the mean accuracy.

Table 1. Summary of data values obtained from records of an intercomparison programme, for estimation of precision and inaccuracy

V_R (interlaboratory comparison value)	V_L (laboratory value)	Differences: $d_n = V_{Rn} - V_{Ln}$	Relative differences (accuracies): $A_n = d_n/V_{Rn}$
V_{R1}	V_{L1}	$d_1 = V_{R1} - V_{L1}$	d_1/V_{R1}
V_{R2}	V_{L2}	$d_2 = V_{R2} - V_{L2}$	d_2/V_{R2}
⋮	⋮	⋮	⋮
V_{Rn}	V_{Ln}	$d_n = V_{Rn} - V_{Ln}$	d_n/V_{Rn}

Table 2. Precision and inaccuracy values obtained for Pb determination in whole blood over the microrange considered (25–35 μg Pb/dL)

No.	E.C. code	V_L (μg/dL)	V_R (μg/dL)	S.D.	Lab no.	$d_n = (V_R - V_L)$	A_n (%)
1	O-723	33.6	33.2	3.5	31	−0.4	−1.20
2	N-725	27.4	27.6	2.9	29	0.2	0.72
3	F-733	32.0	33.6	3.2	32	1.6	4.76
4	Jn-744	24.8	26.4	3.4	34	1.6	6.06
5	O-756	29.0	28.6	4.0	25	−0.4	−1.40
6	Ab-774	26.6	29.5	3.7	31	2.9	9.83
7	O-787	27.2	28.5	4.0	33	1.3	4.56
8	E-796	34.3	34.0	4.0	32	−0.3	−0.88
9	Mr-803	31.0	30.1	2.8	31	−0.9	−2.99
10	Ab-805	31.5	34.2	4.1	30	2.7	7.89
						Mean: 0.83	Mean: 2.73
						S_d: 1.37	S_A: 4.44

Method: ETAAS with Zeeman correction. Intercomparison programme: PICC-PbS (see text).

(6) Calculation of precision as reproducibility, the standard deviation of the accuracies (S_A).

For estimation of these parameters the data can be grouped in a table, as shown in Table 1, and the calculations performed with a spreadsheet program such as Microsoft Excel.

Results and discussion

The precision and inaccuracy values obtained over the microrange considered (25–35 μg/dL) are listed in Table 2. Mean V_L did not differ significantly from mean V_R ($t = 1.917$, $p < 0.05$). Precision and inaccuracy were 4.44% and 2.73%, respectively: both values are thus lower than 5%, highly satisfactory in view of requirement for precision and inaccuracy to be less than 10%.

In this study a new methodology for validation of determination of lead is used; fast, simply and economical. It does not interrupt the routine of the laboratory.

It is not possible to compare the results reported by the use of the information from programmes of interlaboratory comparison or external quality control because there is no estimation of parameters of validation by this new method in the bibliography.

In this line of investigation it is necessary to become aware of ISO 15189 for quality and security reasons for members of the laboratory. Frequently, Biochemical Laboratories provide these values of parameters of validation to members of equipments of clinical investigation and for scientific publications.

Conclusion

This study demonstrates the value of using historical data from intercomparison programmes for revalidation of an analytical method, here a method for determination of Pb in blood, within a range relevant for clinical determinations.

This novel methodology avoids the need to disrupt routine work in order to carry out validation trials and establish validation parameters by classical methodology. The laboratory's daily work is not affected, and it is not a problem if validation trials have not been performed previously at that laboratory.

References

[1] Asociación Española de Normalización y Certificación. Laboratorios clínicos. Requisitos particulares para la calidad y la competencia UNE-EN ISO 15189: 2003. Madrid: AENOR; 2003.
[2] Burnett D. Una guía práctica para la Acreditación del Laboratorio Clínico. Barcelona: SEQC; 2002.
[3] Política de ENAC sobre Intercomparaciones NT-03 Rev. 3 Octubre 2005.
[4] Asociación Española de Normalización y Certificación. Requisitos generales relativos a la competencia de los laboratorios de ensayo y calibración. UNE-EN ISO/IEC 17025: 1999. Madrid: AENOR; 1999.
[5] NCCLS. Procedimientos analíticos para la determinación de plomo en sangre y orina; Guía aprobada. Traducción editada por la SEQC, Pennsylvania, 2001, ISBN 1-56238-437-6.
[6] Herrero E, Serrano E, Sánchez Clavín T, Coperías JL. Plomo. En: Elementos traza: Aspectos bioquímicos, analíticos y clínicos. Cocho JA, Escanero JF, y González-Buitrago JM, editors. Ediciones de la SEQC, Barcelona, 1998. p. 537–58.
[7] Osterloh JD, et al. Quality control data for low blood lead concentrations by three methods used in clinical studies. J Anal Toxicol 1990;14:8–11.
[8] Pearson PJ, Slavin W. A rapid Zeeman graphite furnace atomic absorption spectrophotometric method for the determination of lead in blood. Spectro Chem Acta 1993; 48:925–39.