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COMPARABLE LABORATORY PERFORMANCES IN THE ANALYSIS OF LEAD IN CONTROL SAMPLES AND IN FRESH HUMAN BLOOD

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Summary. - *The validity of quality control programs is based on the assumption that the control samples can be commuted with the real samples, so that, according to the results obtained by a given laboratory on control samples, it is possible to produce reasonable predictions about the reliability of results obtained on real samples. During the implementation of a program of biological monitoring of the general population against the risk of saturnism, a great number of data have been collected allowing the evaluation - as far as blood lead determination is concerned - of the real predictive power of the results obtained by a laboratory during a quality control program, according to its specific level of analytical reliability. The results obtained by eleven laboratories in the analysis of control samples have been compared, by regression analysis, with the results obtained on the same samples by the reference laboratory. The same procedure has been adopted for the results obtained by each laboratory and by the reference laboratory in the duplicate analysis of about 10% of the real samples collected by each center during the biological monitoring program. The comparison between the regression parameters obtained, in both cases, for each laboratory has not produced evidence of systematic differences. Furthermore, a non-parametric evaluation of the data (based on the magnitude of the differences between the results of the laboratories and the results of the reference center) shows, in most cases, similar laboratory performances in the analysis of control samples and of real samples.*

Riassunto (Confrontabilità delle prestazioni dei laboratori nell'analisi del piombo ematico in campioni di controllo e in campioni reali). - *La validità di programmi di controllo di qualità si basa sull'assunto che i campioni di controllo siano commutabili con i campioni reali, così che, in base ai risultati ottenuti da un laboratorio sui campioni di controllo, sia possibile fare ragionevoli previsioni sull'affidabilità dei risultati ottenuti analizzando campioni reali. Durante l'attuazione di un programma di monitoraggio biologico della popolazione contro il rischio di saturnismo sono stati raccolti numerosi dati che permettono di valutare, per quanto riguarda la determinazione del piombo nel sangue, le reali capacità predittive dei risultati ottenuti da un laboratorio nel corso di un programma di controllo di qualità rispetto al suo maggiore o minore livello di affidabilità analitica. I risultati ottenuti da undici laboratori analizzando campioni di controllo sono stati confrontati mediante l'analisi della regressione con quelli ottenuti sugli stessi campioni dal laboratorio di riferimento. Analogamente è stato fatto per i risultati ottenuti da ciascun laboratorio e dal laboratorio di riferimento analizzando in duplicato circa il 10% dei campioni reali raccolti da ciascun centro per l'esecuzione del programma di monitoraggio biologico. Il confronto tra i parametri delle regressioni ottenute in entrambi i casi per ciascun laboratorio non ha messo in evidenza differenze sistematiche. Anche una valutazione non parametrica dei dati (in base all'ampiezza degli scarti fra i risultati dei laboratori e quelli del laboratorio di riferimento) evidenzia, nella maggior parte dei casi, analoghe prestazioni del laboratorio nell'analisi dei campioni di controllo e nell'analisi dei campioni reali.*

Introduction

Since a few years, an Interlaboratorial Quality Assurance Programme (IQAP) for blood lead levels determination (PbB) has been promoted in Italy by a working group of the Istituto Superiore di Sanità (Italian National Institute of Health).

The adopted scheme consisted of analyses of samples with target value (internal quality control) and periodical analyses of samples with lead concentrations unknown to the analysts (external quality evaluation).

Although the procedures adopted for the preparation and distribution of the control samples and for the elaboration and evaluation of the results [1-3] provided a reasonable warranty that the quality of the performance of the laboratories on control samples agreed well with the one obtained on fresh human blood, some doubt could persist, particularly considering that control samples were prepared from materials of animal origin and especially treated in order to assure their homogeneity, stability and preser-

vation. As a consequence, their analytical behaviour can be different from that of samples of fresh human blood, and usually they show greater analytical problems.

The possibility to evaluate the real predictive value of the results obtained by a laboratory during an IQAP by comparing them to the analytical performance in determining lead in samples of fresh human blood has occurred during the implementation of a program for the biological monitoring of the risk of saturnism in the Italian population [4, 5].

On this occasion, eleven laboratories, already participating in the IQAP, carried out the analytical operations for the multicentric screening campaign, during which, on the basis of a previous European experience [6], about 10% of the blood samples examined in each operative center had also been analyzed by the IQAP coordinative center, chosen as the reference center (RC).

The results obtained by the eleven laboratories, both during the quality assurance program and the analysis of fresh human blood samples, are here compared and discussed.

Materials and methods

Fresh human blood samples were collected from healthy subjects in different Italian regional centers, during the monitoring program. About 10% of the collected samples were subdivided into two aliquots, one of which was analyzed by the laboratory of the operative center, and the other sent to the RC.

All the participating laboratories used graphite furnace atomic absorption spectrometry (GFAAS) for blood lead determination. Details about the analytical method adopted by each laboratory are reported in Table 1.

The quality assurance procedures and schemes have been described in detail elsewhere [1-3, 7].

Results and discussion

The analytical performance of the eleven laboratories and of the RC, at the end of each year of IQAP activity, can be evaluated from the percentage of unacceptable results obtained by each of them, according to the adopted acceptability criterion (Table 2). It can be observed that, in most cases, a progressive improvement followed the adhesion to the program. On the contrary, the performance of the RC might appear rather discontinuous, but, as the only unacceptable results obtained by the RC were on samples at concentration levels lower than 50 μg/l, this apparent contradiction will be explained by the following considerations.

With the aim to evaluate more thoroughly the analytical agreement between observed and expected values, with particular attention to the lead concentrations within the range expected for the general population, the parameters of the regression lines between the results obtained by each laboratory during the entire period of participation in the IQAP (y), and the median values of the results obtained for each control sample by all the laboratories (x) were calculated, taking into account only the control samples at lead concentration ≤ 350 μg/l (Table 3). In all cases, the observed slopes are close to the unit, intercept values are greater than 10 μg/l (as absolute value) only in three cases, and standard errors of the estimate are almost always within the range 10-20 μg/l. In most cases, the determination coefficients show a good agreement between the results of each laboratory and the median values. Nevertheless, attention should be paid to the fact that a constant difference exists between the RC values and the median values (Table 3; Fig. 1), as pointed out by the significant intercept value (-19.14 μg/l). Even though such a difference could be due to a poor performance of the RC, in our opinion it seems more reasonable to ascribe it to a general tendency to overestimate from most participating laboratories. It is known, in fact, that the downward trend in blood lead levels, generally observed in recent years, has been often ascribed to the improvement of the analytical determinations [8].

To confirm this hypothesis, we report in Table 4 the results obtained by carrying out, for a group of control samples prepared from the same material, the regression analysis between the lead amounts added to the bovine blood (x) and the values obtained both by two research laboratories (RC and Joint Research Center of Ispra) and two representative laboratories of the operative centers. The intercept value is an estimate of the "natural" lead content of the bovine blood used for sample preparation, for which the isotopic dilution mass spectrometry (IDMS) analysis yielded the value of 11 μg/l.

Although substantial differences are not observed among the slopes (in all cases near to the unit), the analytical performance of the two research laboratories should be considered as different from that of the two operative centers.

In fact, whereas in all cases the values of the determination coefficients point out a linear trend, the standard errors of the estimate of the two operative centers result more than three times as high than those obtained by the two research laboratories. Furthermore, the intercept values of the two operative centers are comparable (and the same is true for the two research laboratories), but result significantly higher (about twice as much) than those obtained by the two research laboratories.

Since the lead values observed by the two research laboratories in non-supplemented bovine blood are rather close to those estimated by IDMS, the high values of the intercept of the regression lines concerning the results of the two selected operative centers are likely due to a true tendency to overestimate low lead levels in non-supplemented blood. This fact would explain the observation that, in the regressions between the results of the laboratories and those of the RC, a positive intercept - approximately of the same magnitude as the difference between the means of the intercepts of the two pairs of laboratories considered above - is almost constantly seen.

Table 1. - *Analytical methods adopted by the eleven laboratories and the reference center*

Laboratory	Instrumentation (Perkin-Elmer)	Injection *(a)*	L'Vov platform *(b)*	Matrix modifier *(c)*	Sample pretreatment	Calibration *(d)*
RC	Zeeman/5000 + HGA 500	A	+	+	dilution	1
1	3030 + HGA 600	A	-	-	dilution	1
2	460 + HGA 400	A	-	-	dilution	2
3	3030 + HGA 600		+	+	dilution	1
4	272 + HGA 500	A	-	-	dilution	2
5	306 + HGA 76B	M	-	-	extraction	1
6	603 + HGA 2100	A	-	-	dilution	1
7	2380	M	-	-	dilution	3
8	430 + HGA 2100	A	+	-	dilution	3
9	420 + HGA 76B	A	-	-	dilution	1
10	3030 + HGA 600	A	-	-	dilution	1
11	272 + HGA 400	A	-	-	dilution	3

(a) manual (M) or automated (A) injection
(b) graphite furnace with (+) or without (-) L'Vov platform
(c) use of matrix modifier: any (+), none (-)
(d) calibration procedures: 1 = standard solutions in blood matrix; 2 = aqueous standard solutions; 3 = internal addition method
RC = reference center

Table 2. - *Percentage of unacceptable results provided by the laboratories during each phase of the quality assurance scheme*

Laboratory	I phase	II phase	III phase	IV phase
RC	7.5	18.8	9.4	6.3
1	22.7	25.0	50.0	6.3
2	25.6	0.0	12.5	6.3
3	67.0	47.5	-	37.5
4	20.0	10.4	6.3	0.0
5	27.1	37.5	6.3	12.5
6	45.0	52.1	-	-
7	2.1	0.0	6.3	6.3
8	20.8	6.3	6.3	3.1
9	35.4	8.3	37.5	12.5
10	-	20.0	0.0	0.0
11	87.5	29.2	3.1	6.3

RC = reference center

Table 3. - *Parameters of the regression lines obtained by comparing the results provided by each laboratory during the quality assurance scheme (y) with the medians of the results of all the participating laboratories (x)*

Laboratory	n	b	a	SE	r^2
RC	86	1.0877	- 19.14	10.12	0.9909
1	87	0.9863	3.68	23.50	0.9496
2	77	0.9727	6.66	20.35	0.9626
3	44	1.1017	- 20.54	42.60	0.8551
4	78	1.0112	- 3.77	14.57	0.9729
5	90	0.9852	7.18	23.49	0.9389
6	56	1.0926	- 8.83	35.40	0.8986
7	66	0.9592	8.42	16.52	0.9674
8	92	1.0256	- 7.45	12.23	0.9866
9	84	0.9682	6.74	26.86	0.9252
10	54	1.0399	0.09	17.43	0.9711
11	58	0.9567	12.03	16.01	0.9726

n: number of samples analyzed by the laboratory
b: slope
a: intercept, µg/l
SE: standard error of the estimate, µg/l
r^2: determination coefficient
RC = reference center

Table 4. - *Parameters of the regression lines obtained by comparing the lead amounts found by four laboratories (two research and two operative centers) with the lead amounts added to various fractions of the same bovine blood*

Laboratory	n	b	a	SE	r^2
JRC	19	0.9660	32.06	5.70	0.9979
RC	21	1.0068	25.11	4.70	0.9985
1	20	0.9613	47.72	16.67	0.9819
2	19	1.0016	41.00	20.52	0.9796

n: number of samples analyzed by each laboratory
b: slope
a: intercept, µg/l
SE: standard error of the estimate, µg/l
r^2: determination coefficient
JRC = Joint Reseach Center (Ispra)
RC = reference center

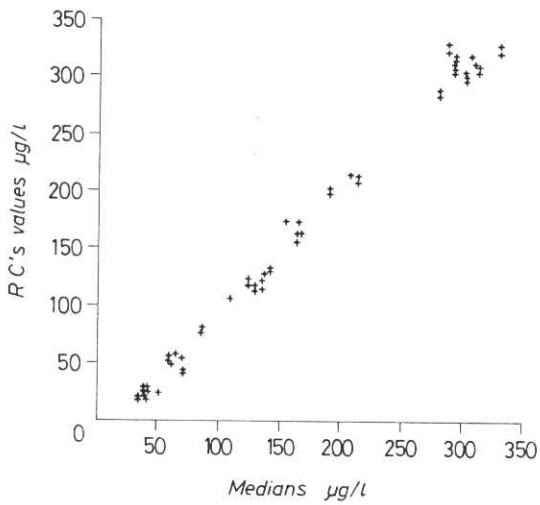

Fig. 1. - Regression between the values obtained by the RC during the IQAP (y) and the median of the values measured for each control sample by all the laboratories.

On the basis of the data obtained by the IQAP and by the parallel analyses performed by the operative centers and the RC on the same samples of human blood, we evaluated by regression analysis the performance of eleven laboratories, both on control and human blood samples, as compared with that of the RC.

Table 5 shows the parameters of both regressions for each laboratory. The slopes observed are not statistically different in six cases ($p > 0.20$ in five cases). In the other cases, in which differences between slopes are statistically significant, a systematic trend cannot be pointed out: in certain cases the slope of the regression line for the human blood samples is higher than that of the control samples; in other cases the opposite is true. The intercept observed values - for each laboratory, in both cases - substantially agree (except that for two laboratories) and the determination coefficients are always close to the unit.

Furthermore, to support the assumption that the use of control samples can effectively monitor the analytical procedures on real (human blood) samples, it must be pointed out that Table 5 does not show any systematic pattern also for the other parameters computed on real samples and, correspondingly, on control samples. For instance, in six cases the intercept is greater for the control samples while in five cases is greater for the real samples; the standard errors of the estimate show a similar pattern (six cases *vs* five). Besides, differences are almost always very slight and the differences between the determination coefficients are also rather slight.

An example (laboratory 4) pointing out the good agreement between the analytical performances obtained on control and on real samples is shown in Fig. 2.

On the other hand, in certain cases, the differences observed could be partly explained by considering that the human blood samples had a narrower range of lead concentration levels than the control samples and that their analysis was carried out in a shorter period.

Also it must be kept in mind that the great amount of available data gives statistical significance even to small differences.

Finally, a few considerations can be done by looking at the simplified evaluation of laboratory performance in the analysis of real samples. Table 6 shows the percentage of results - obtained by each laboratory - in which the difference from the RC values for the same samples, exceeded 30 and 40 µg/l, respectively: 30 µg/l is approximately the average value of the maximum accepted differences for concentrations ranging from 50 to 350 µg/l according to the acceptability criterion adopted during the IQAP. The percentage of results exceeding by > 40 µg/l the corresponding values measured by the RC has been considered here, so that a comparison with a similar evaluation of the performances of European laboratories participating in a population surveillance campaign against the risk of saturnism is possible [9].

The comparison between Table 6 and Table 2 shows that, substantially, the performances in the analyses done on control samples (Table 2) have the same degree of reliability of those done on real samples. Only in one case (laboratory 3) a marked improvement, due to the substitution of obsolete equipment, is present. Only in one case (laboratory 9) performances have markedly - and quite inexplicably - worsened.

Table 5. - *Parameters of the regression carried out by comparing the results provided by the RC (x), analyzing control and fresh human samples with those obtained by each operative center (y) on the same samples*

Laboratory	S	n	Range µg/l	b	Student's t-test	a µg/l	SE µg/l	r^2
1	H	97	50-400	0.9168	(1.26 n.s.)	24.44	18.23	0.8674
	C	64	20-350	0.8477		30.06	29.07	0.9190
2	H	21	90-500	0.9675	(4.39*)	20.27	18.94	0.9612
	C	46	20-350	0.8656		30.95	20.10	0.9583
3	H	130	50-330	0.8440	(4.57*)	15.24	17.48	0.8565
	C	28	50-350	1.0574		- 5.85	30.58	0.9276
4	H	68	50-300	1.0141	(2.73**)	18.71	25.36	0.8165
	C	48	20-350	0.9338		17.07	16.61	0.9640
5	H	49	50-350	0.9495	(1.06 n.s.)	17.33	24.99	0.8815
	C	52	20-350	0.9201		24.24	24.83	0.9394
6	H	38	50-250	0.8221	(3.61*)	32.50	23.02	0.7482
	C	38	20-370	0.9882		12.92	38.16	0.8871
7	H	150	50-200	0.8995	(0.90 n.s.)	22.76	16.27	0.7110
	C	46	20-370	0.9160		15.37	12.73	0.9850
8	H	32	50-300	0.9413	(0.21 n.s.)	15.28	17.34	0.9170
	C	66	20-350	0.9446		11.44	14.53	0.9824
9	H	263	50-500	0.9559	(1.84 n.s.)	15.49	26.34	0.7974
	C	54	20-370	0.9146		24.57	27.18	0.9294
10	H	97	50-350	0.9856	(1.13 n.s.)	4.22	10.49	0.9608
	C	37	20-320	0.9869		7.95	10.13	0.9892
11	H	106	50-350	1.1319	(11.44*)	2.95	11.65	0.9545
	C	34	20-350	0.9305		26.09	17.15	0.9683

S: human (H) or control (C) samples
n: number of samples
b: slope
a: intercept
SE: standard error of the estimate
r^2: determination coefficient
* = $p < 0.001$; ** = $p < 0.01$

Table 6. - *Average blood lead levels obtained by each laboratory and by the RC in the analysis of the same group of human blood samples and percentage of the differences > 30 µg/l and > 40 µg/l, respectively, obtained by each laboratory when compared to the values measured by the RC*

Laboratory	n	Mean value µg/l		Results (%) with differences to the RC values	
		Laboratory	RC	> 30 µg/l	> 40 µg/l
1	98	113	98	16.3	8.1
2	21	180	165	9.5	9.5
3	130	119	123	6.1	5
4	68	140	120	38.2	23.5
5	49	127	116	12.2	4.1
6	38	141	132	13.1	7.9
7	150	93	78	10.6	7.5
8	33	165	157	9.1	6
9	263	126	116	23.9	13
10	97	101	99	2.1	2.1
11	106	120	103	7.5	5.7
A*	40	189	151	60	47
B*	153	131	138	55	31

* These laboratories had not participated in the IQAP
RC = reference center

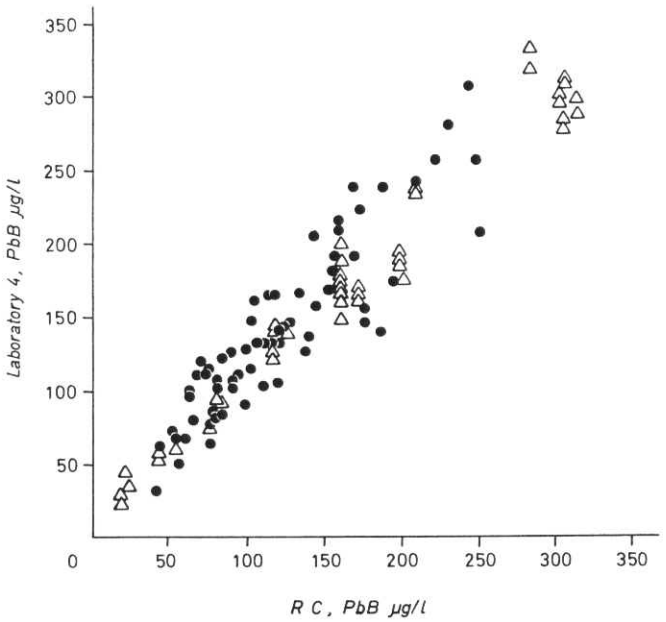

Fig. 2. - Regressions between the results of the RC (x) and those of laboratory 4 (y) obtained on control (full circle) and human blood samples (triangles), respectively.

Furthermore, a tendency of the laboratories to overestimate is confirmed.

The data from laboratories A and B, that had not followed an adequate quality control procedure, present a high degree of unreliability: this fact once more points out the need, during the implementation of multicentric screening campaigns, to promote interlaboratorial comparisons that might reduce the analytic variability.

Finally, the analytical reliability of the eleven Italian laboratories considered, that show an 8.2% of results with differences > 40 µg/l when compared to the RC values, is similar to that of the European laboratories (average percentage of 8.3) participating in a population surveillance campaign against the risk of saturnism [9].

Conclusions

In order to verify the predicting value of the results obtained during an Interlaboratorial Quality Assurance Programme for blood lead determination concerning the actual performances of the participating laboratories, we compared the results obtained by eleven laboratories both on control materials and fresh human samples -collected during a multicentric screening campaign - with those obtained by the laboratory chosen as reference center (at the Istituto Superiore di Sanità). The comparison between the parameters of the regression lines obtained in both cases did not reveal significant differences, neither systematic trends.

Also a non-parametric analysis of data (performed on the magnitude of the differences between the results of the operative centers and those of the reference center) shows, in most cases, similar performances in the analysis of control samples and in the analysis of real samples.

We can conclude that, at present, for blood lead determination, the results obtained in a properly designed quality assurance programme are representative of the actual performance of the laboratory.

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