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The network of the Italian laboratories: a proficiency test on the quantification of trace elements in serum

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Summary. - A proficiency test on the quantification of Al, Cu, Mn, Se and Zn in serum was carried out to verify the performance of about 30 regional laboratories of the network of Italian laboratories. The exercise consisted of four runs in which the laboratories were free in choosing analytical methods to determine trace elements in freeze-dried animal serum. Laboratories performances were evaluated by the study of statistical functions as Coefficients of Variation (CV), Youden plot and Z-score value. As for Al, the results were generally characterized by good accuracy and precision, in spite of the low levels of the element (5-7 μg l^{-1}). Copper determination had some problems only at low concentration (about 160 μg l^{-1} - first run), in which an elevated number of anomalous data were found. Better data were achieved for Zn, for which anomalous values were mainly stragglers than outliers. Due to the low number of data for Mn (concentrations from 0.6 to 60 μg l^{-1}) and Se (concentration from 45 to 106 μg l^{-1}), a restricted statistical treatment was applied; for these elements high CV values were found (range from 30 to 80%). The results of this trial confirmed that participation in a proficiency test represents a benefit for all analytical laboratories. In fact, with few exceptions, most of the participants improved their performances in terms of Z-score values.

Key words: proficiency test, trace elements, biological fluid, serum.

Riassunto *(La rete dei laboratori analitici italiani: un controllo di qualità esterno per la quantificazione degli elementi in traccia sul siero)*. - È stato effettuato un esercizio analitico interlaboratoriale per la determinazione di Al, Cu, Mn, Se e Zn in siero, al fine di verificare le prestazioni analitiche di circa 30 laboratori periferici del Servizio Sanitario Nazionale. Il programma è stato articolato in quattro fasi, durante le quali ogni laboratorio aveva la possibilità di scegliere il metodo di determinazione analitico da applicare. L'intero esercizio è stato valutato statisticamente attraverso il coefficiente di variazione (CV), il diagramma di Youden e lo Z-*score*. Nel caso dell'Al, i risultati sono caratterizzati da una buona accuratezza e precisione nonostante l'intervallo di concentrazione risultasse molto basso (5 - 7 μg l^{-1}). La determinazione del Cu presenta alcuni di dati anomali soprattutto alla bassa concentrazione del primo invio (160 μg l^{-1}μg). Risultati migliori sono stati ottenuti per lo Zn, per il quale la maggior parte dei valori risultavano essere anomali più che estremi. Per il Mn e Se, a causa del basso numero di risultati inviati, è stato applicato un trattamento statistico limitato; il CV, per questi due elementi, risultava essere elevato tra il 30 al 80%. I risultati ottenuti confermano che tali esercizi producono benefici per tutti i laboratori partecipanti. Infatti, con poche eccezioni, la maggior parte degli aderenti all'esercizio ha migliorato le proprie prestazioni in termini di Z-*score*.

Parole chiave: controllo di qualità esterno, elementi in traccia, fluidi biologici, siero.

Introduction

Validation is a general concept sustaining the quality of the analytical results. This includes the initial assessment of performance characteristics, several types of inter-laboratory testing and quality control. As concern the inter-laboratory studies, they are, generally, carried out as experiments among several laboratories in order to ascertain some requirements, e.g. the concentration assessment of an analyte in one or various samples, the bias evaluation of a method (method bias) or a laboratories (laboratory bias) as well as the certification for reference materials. These comparative experiments, commonly called "proficiency tests" or "round robin study", are also essential part of requirements for laboratory accreditation.

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Proficiency tests are, thus, a series of experiments to check the performance of laboratories to quantify an analyte, or a group analytes, in a well-definite matrix [1-3]. The participating laboratories are usually free in the choice of the test methods, but they ought to prove their technical competence regularly, i.e., several times a year [4, 5]. The number of test material will principally depend on the range of concentration that needs to be covered. For methods that have to cover a concentration range, at least analyses at the lowest and the highest levels are suggested. Although the protocol does not require a minimum number of participants for the proficiency test, a minimum number of eight laboratories and a typical number of participants ranging from 15 to 30 is recommended [6].

In this context, a collaborative study on trace elements determination in serum was organized as a part of the overall project named: "Role of the neurotoxic chemical elements and of the genetic factors in the aetiology of neuro-degenerative diseases". The aim of this proficiency test was the implementation of analytical performances of the Italian network chemical laboratories involved in the health population surveillance from both clinical and occupational point of view.

Experimental

In order to obtain a statistically significant treatment of the final outcome, 32 regional laboratories, amongst hospitals, institutes of occupational medicine and university clinics, took part in the proficiency test. The whole exercise, consisting of four runs, was characterized by a rather satisfactory number of laboratories, especially for the first and the second run (Table 1). At first, participants were asked to choose one or more elements of interest, among those proposed by the organizers, namely: Al, Cu, Mn, Rb, Se, V and Zn. The subsequent trials were solely focused on Al, Cu, Mn, Se and Zn. The full list of the structures recruited is reported in Annex 1, whilst the regional distribution of the laboratories is depicted in Fig. 1.

Serum was selected as the most suitable biological fluid to use in biological monitoring and for diagnostic intents. Animal serum was considered for the execution of the programme for safety and practical reasons. Physical and chemical characteristics and elements concentration were also taken into account to select the fit for purpose animal serum. In particular, the elements level in this animal serum should be the closest to the human one. Therefore, serum deriving from large size animals, like bovines and equines, was considered more appropriate. Moreover, high content in haemoglobin was assessed as unacceptable, because not comparable with those normally found in human sera. Thus, serum purchasable from a zoo technical firm was preferred instead of those from industrial units, since the last provided only serum destined to cells culture with elevated haemoglobin level (> 20 mg 100 ml^{-1}). For preparation and delivery of serum aliquots, defined criteria and appropriate methodologies were followed in order to preserve the analytical information of the exercise samples.

Table 1. - Scheme of the intercomparison exercise

	Runs (no.)			
	I	II	III	IV
Date	February 2003	June 2003	November 2003	July 2004
Sample provided (no.)	1	2	2	2
Laboratories (no.)	32	30	25	23
Results supplied (no.)	29	22	19	16

Fig. 1. - Number of participant laboratories per Region.

Decontamination of the serum vials

Vials for the serum were selected in order to guarantee: *i)* maximum resistance to freezing (up to -80 °C); *ii)* minimum release and absorption of elements of interest; and, *iii)* simple decontamination procedures. High-density polyethylene vials (Packard Bioscience Company, Groningen, The Netherlands), intended for scintillation techniques, showed satisfying resistance at low temperatures and mechanical properties. A simple decontamination procedure was set up by means of a series of rinsing with high purity deionised water (C < 0.3 μS cm^{-1}) (Water Purification Systems, Labconco Corp., Kansas City, MO, USA). Afterward, the decontaminated containers were upturned, covered by filter paper and left to dry at room temperature.

Fifty vials were preliminarily checked for the contamination by the following procedure: vials were filled with a solution of HNO_3 0.015 N Suprapur (Merck, Darmstadt, Germany), sealed, shaken for 2 hrs and then left still for 2 days; finally, an additional phase of 2 hrs shaking was performed. The obtained contact solutions were analysed by means of sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) (Element 1, Thermo-Finnigan, Bremen, Germany) for the level of Al, Cu, Mn, and Zn. The results turned out to be reasonably low: Al < 0.82 μg l^{-1}, Cu < 2 μg l^{-1}, Mn < 0.10 μg l^{-1} and Zn 10.0 μg l^{-1}. In the case of Zn, the amount found, although rather high, could be considered negligible when compared to the zinc level in serum of the exercise, ranging from 600 to 1000 μg l^{-1}.

Sample pre-treatment and freeze-drying

To establish the stability of serum after the dilution in deionised water, several attempts were conducted at the ratio 1:2, 1:4 and 1:10. It was observed that the mere addition of water caused a precipitate, which hampered the subsequent elements quantification. The addition of NaCl stabilized the serum during the dilution for the time required for the analysis i.e., 5 or 6 hrs. The whole quantity of serum was, then, added by NaCl Suprapur (Merck, Germany) to reach a final concentration of 1%, homogenized and sub sampled into the clean vials.

Aliquots of about 5 ± 0.02 g of serum were transferred into the vials by weighing. This allowed achieving repeatability in the sub sampling of about 0.03 %. Closed vials containing the serum were kept at - 80 °C for at least 48 hrs before starting the freeze-drying process, carried out through the application of four steps of 2, 4, 15 and 3 hrs, respectively, with temperature progressively varied from - 40 °C to + 25°C, and the pressure from 1.016 mbar (first step) to < 0.1 mbar (steps 2, 3, 4) . The elements concentration was quantified in serum before the freeze-drying process; these levels were similar to those of the human serum. The concentration in serum was modified in some cases during the exercise, by adding known amounts of stock standard solutions of 1000 μg ml^{-1} (Spex Industries Inc., Edison, NJ, USA). Serum was spiked with the lowest volume of standard solution to minimize the matrix composition change.

The yield factor of the freeze-drying was calculated, in terms of mean and its standard deviation s_x, by comparing the weight of each vial before and immediately after the process [7]. Data reported in Table 2 reveal that the residual serum percentage in each vial after the freeze-drying was in the range of 3.87 - 9.70% as function of the nature and composition of animal serum. In fact, the same freeze-drying procedure applied on serum of identical lot number produced similar yield factors and s_x, taken in different period of time.

The homogeneity of the prepared samples was verified by determining the analytes in 1/10 specimens of each lyophilised serum lot. Measurements were performed on randomly selected samples by SF-ICP-MS, obtaining reproducibility never statistically different

Table 2. - Mean yield factors of the freeze-drying

Run	Level	Yield factor (%)	s_x (%)
I	1	5.70	0.032
II	1	8.45	0.016
	2*	3.99	0.019
III	1*	3.87	0.019
	2°	9.29	0.012
IV	1°	9.51	0.012
	2	9.70	0.012

Sera with the same mark were identical.

Table 3. - Number of participants and replies per run

Run	Participants (no.)	Replies (no.)					
		Al	Cu	Mn	Se	Zn	Total
I	32	22	25	17	8	24	29
II	30	17	17	10	7	19	22
III	25	15	16	9	6	17	19
IV	23	12	13	6	3	14	16

from the repeatability. In fact, the variation coefficient (CV) was generally found to be below 10%, even in the case of spiked serum, although the small volume added to the serum could limit the uniform dispersion of the spike on a great mass and rise absorption phenomena on proteins at the moment of the addition. It can be inferred that the material prepared was definitely appropriate for the aims of the intercomparison exercise.

Results and discussion

The number of participants and of incoming replies has been decreasing during the exercise time, as reported in Table 3. Depending on the elements sorted out by laboratories for each exercise, the number of answers obtained was also diminishing. Percentage distribution of techniques used by the laboratories participants is displayed in Fig. 2. Electrothermal atomic absorption spectrometry (ETA-AAS) and flame atomic absorption spectrometry (F-AAS) were the techniques mostly used.

Determination of Al was mainly carried out by ETA-AAS, the Zeeman system (55%) was preferred against the deuterium correction (20%); instead, ICP-MS and inductively coupled plasma atomic emission spectrometry (ICP-AES) were employed in the 10% and 5% of the cases, respectively. Copper was preferably measured by AAS (74%), only three laboratories (26%) resorted to ICP-AES and ICP-MS. As regards Mn, Z-ETA-AAS (39%), ETA-AAS (4%) and/or F-AAS (35%) were considered the most suitable techniques, while, only 9% and 13% of participants elected ICP-AES and ICP-MS, respectively. Selenium was found to be at low level and this justified the utilization of ETA-AAS (62.5%) and ICP-MS (37.5%). Finally, the high contents of Zn in serum allowed the extensive employment of F-AAS (63%).

The complete results of the interlaboratory trial are reported in Tables 4 and 5, in which mean, standard deviation, median, percent CV and confidence interval (CI) at 95% are listed. Outliers were identified by the Grubbs test and ruled out from the tables [8]. For Al (Table 4) the mean values were characterised by elevated CV of about 50%. This trend for Al was due to its very low level; in fact, when a spiked sample of 20 µg l^{-1} was provided, the relevant CV decreased below 20%. In consideration of the difficulties encountered in the quantification of Al, the general data obtained by the laboratories was fairly satisfactory. Copper determination (Table 4) had some problems only at low concentration (I run), in which an elevated number of anomalous data (n = 5) and high variation coefficient (40.7%) was found. Instead, for

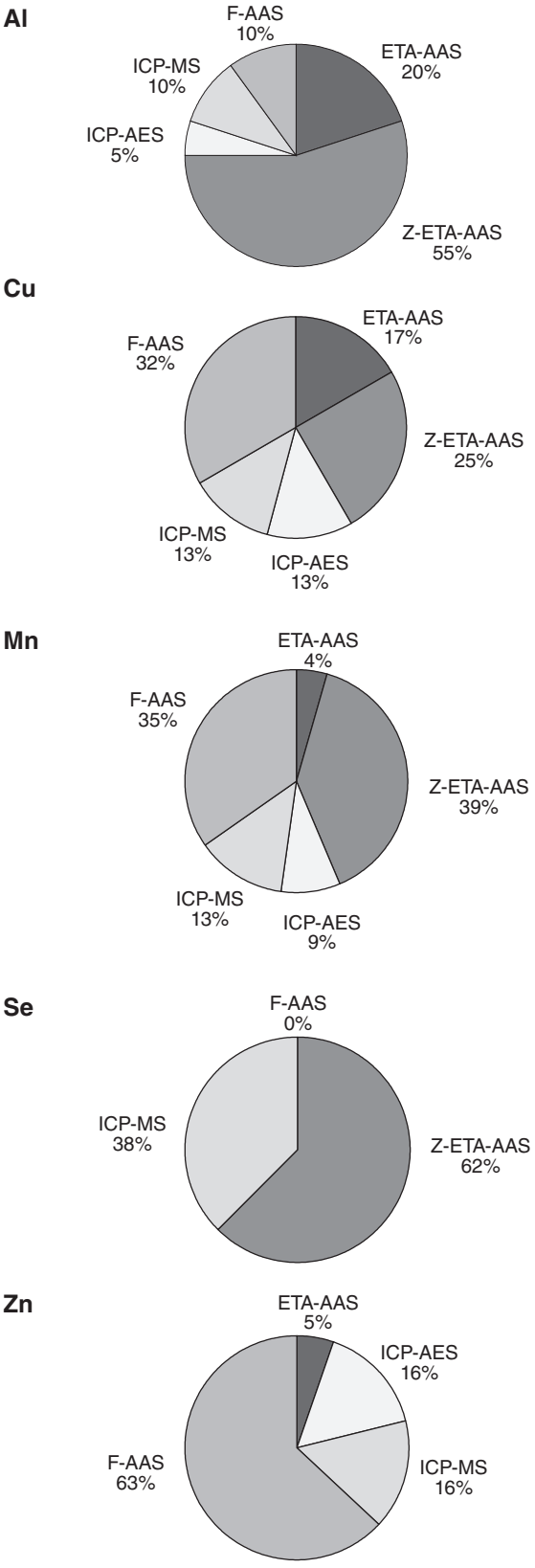

Fig. 2. - Percentage distribution of techniques as used by the laboratories.

Table 4. - Figures of merit of the exercise

Run	Outliers (no.)	Concentration (µg l^{-1})(*)			CV (%)
		Mean ± s_x	Median	CI (95%)	
Al					
I Level 1	1	5.3 ± 2.4	5.0	4.2 - 6.4	45.0
II Level 1	1	6.4 ± 2.5	6.3	5.0 - 7.7	39.0
II Level 2	0	6.6 ± 3.1	7.1	5.0 - 8.2	46.9
III Level 1	1	27.5 ± 4.9	26.7	24 - 30	17.8
III Level 2	0	5.8 ± 2.6	5.3	4.3 - 7.3	44.8
IV Level 1	0	6.5 ± 3.5	5.0	4.3 - 8.7	53.7
IV Level 2	1	9.1 ± 2.2	5.2	3.6 - 6.6	43.1
Cu					
I Level 1	5	159 ± 24	163	147 - 171	15.7
II Level 1	0	1,044 ± 95	1,040	995 - 1,094	9.1
II Level 2	0	404 ± 47	399	380 - 428	11.6
III Level 1	0	418 ± 53	401	389 - 447	12.7
III Level 2	0	1,597 ± 112	1,640	1,532 - 1,662	7.0
IV Level 1	2	1,666 ± 111	1,647	1,591 - 1,741	6.7
IV Level 2	1	1,095 ± 134	1,120	1,014 - 1,176	12.2
Zn					
I Level 1	1	3,210 ± 280	3,220	3,090 - 3,330	8.7
II Level 1	2	769 ± 66	763	735 - 803	8.6
II Level 2	2	339 ± 47	330	315 - 363	13.9
III Level 1	0	315 ± 47	327	290 - 340	14.9
III Level 2	2	910 ± 63	892	875 - 945	6.9
IV Level 1	1	934 ± 69	930	892 - 976	7.4
IV Level 2	2	870 ± 57	878	834 - 906	6.5

(*) Outliers were not included; CV: variation coefficient; CI: confidence interval.

the next runs the results were reliable in terms of variation coefficient and number of stragglers and outliers. Data even better were achieved for Zn (Table 4), with anomalous values characterised for the most part by stragglers than outliers. Due to the low number of data obtained for Mn and Se, Tables 5 reports only mean, s_x and CI. As a consequence of the paucity of data collected, a comprehensive statistical treatment of results for these elements could not be prepared.

Laboratories performances were evaluated by the study of statistical functions showed in the tables above. Furthermore, the Youden plot was elaborated to assess the precision and accuracy of the methods in use by the participant laboratories [9]. The Youden plot is a graphical method to analyse inter-laboratory data, where all laboratories analysed 2 samples. To this end, identical serum samples, with the same elements concentration (because the two samples must be similar and reasonably close in the concentration to be properly evaluated), were shipped to participants in different times. Each point in the plot corresponded to the results of one laboratory and was defined by a first response variable on the horizontal axis (i.e., run 1 or response value) and a second response variable 2 (i.e., run 2 or response value) on the vertical axis. In correspondence of the concentration value assigned, a horizontal median line was drawn parallel to the x-axis and a second median line was drawn parallel to the y-axis, to divide the graph in four areas. On the intersection of the two median lines a circle was drawn. The radius of this circle was the product of the standard deviation (S_r) and a factor (b) that considers the percentage of the theoretical points to be available inside the circle:

Table 5. - Figures of merit of the exercise

Run	Laboratory (no.)	Concentration (µg l^{-1})		
		Mean ± s_x	Median	CI (95%)
Mn				
I Level 1	15	50.5 ± 6.4	48.9	47.1 - 53.9
II Level 1	10	1.63 ± 0.87	1.65	1.00 - 2.25
II Level 2	"	0.64 ± 0.35	0.64	0.35 - 0.93
III Level 1	9	38.3 ± 3.3	38.5	35.2 - 41.3
III Level 2	"	0.91 ± 0.37	1.02	0.60 - 1.22
IV Level 1	6	1.05 ± 0.52	1.01	0.67 - 1.43
IV Level 2	"	2.00 ± 0.60	1.99	1.37 - 2.63
Se				
I Level 1	8	32.6 ± 29	20.4	12.3 - 52.9
II Level 1	7	89 ± 11	94.5	78 - 99
II Level 2	"	44.6 ± 4.6	47.3	40.3 - 48.9
III Level 1	6	54 ± 22	46.0	31 - 77
III Level 2	"	105 ± 33	103	70 - 140
IV Level 1	3	105 ± 6	108	99 - 112
IV Level 2	"	93.8 ± 4.5	95.1	89 - 99

CI: confidence interval.

$$S_r = (\sum d^2/2n)^{1/2}$$

$$b = [-2\ln(1- \%/100)]^{1/2}$$

where d is the difference between the values of the two measurements.

The accuracy was determined by the distance from the centre of the circle and the precision by the distance from the line drawn from the origin to the intersection of the two median lines. In Figs 3 through 5, Youden plots for Al, Cu and Zn are reported.

In the case of Al (Fig. 3), two samples were provided at a level of 5 µg l^{-1} with a consensus values of 5.0 and 5.3 µg l^{-1}. Five laboratories (total was eleven) were found out of the circle; this was due to both systematic and random errors. The remaining results, about 55%, were inside the circle. Four laboratories attained an excellent accuracy and precision, although the low level of the element considered.

As previously stated, determinations of Cu and Zn were characterised by an evident enhancement of the laboratories performances. In fact, the relevant Youden plot for Cu (Fig. 4) at a concentration of 1,000 µg l^{-1} showed 10/11 values (91%) inside the circle, with a good accuracy and precision. Likewise, for Zn (Fig. 5) two different levels in serum were prepared to simulate a pathological lacking (A) and normal value (B). In the first case, most values fell inside the circle, probably as a consequence of a bigger radius, with a fair homogenous distribution in the four areas. This meant that the random errors were predominant. In the case (B), where high concentrations of Zn were found, some values were outside the circle, but again well distributed among the four areas.

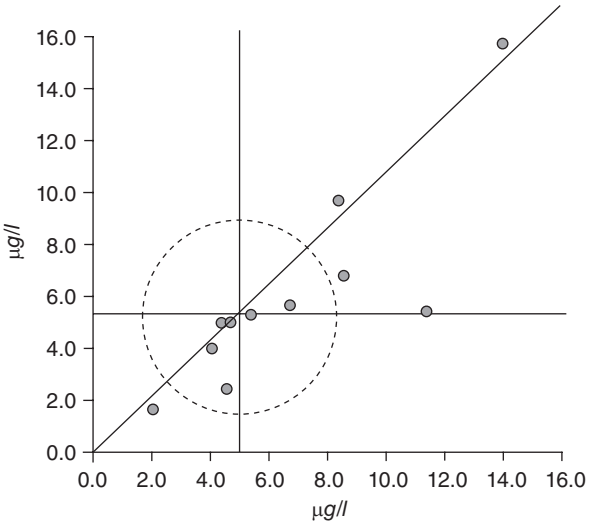

Fig. 3. - Youden plot for Al determination.

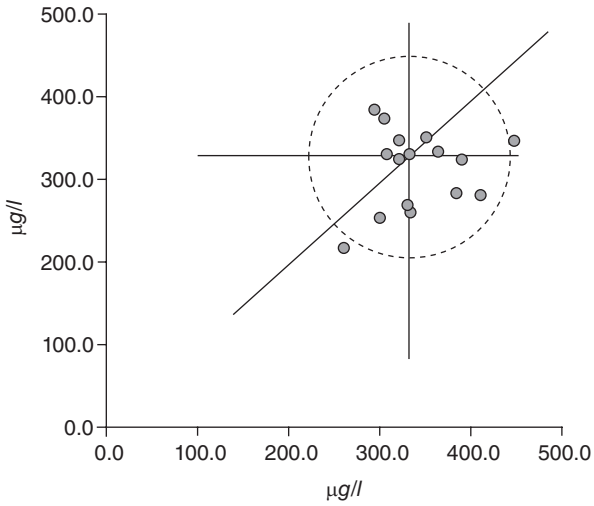

Fig. 4. - Youden plot for Cu determination.

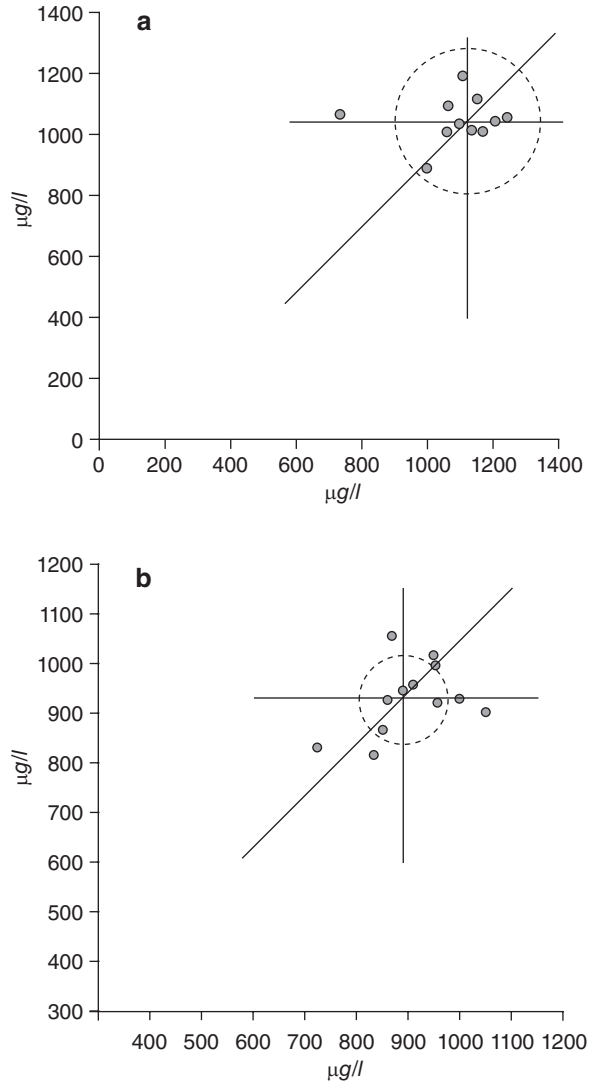

Fig. 5. - Youden plots for Zn determination, for 2 different serum levels (a and b).

In order to evaluate the performance of participants in all runs, the Z-score approach was applied. The Z-score was calculated by using the following expression:

$$Z = (x - X^*)/\sigma$$

where x, was the value supplied by a laboratory; X^*, the target value or the mean of the laboratories; σ, the standard deviation value adopted.

It is clear that the Z value is strictly dependent on σ. The σ factor was selected by considering: *i)* type of matrix, *ii)* analyte level and difficulties in performing the analysis, *iii)* analytical technique available and, *iv)* data quality obtainable. In this regards, the σ factor was chosen according to Horwitz or Thompson formulas, at concentration >120 µg l^{-1} [9, 10] In Table 6, σ values and concentration ranges for the execution of the proficiency test are showed. The performance of the laboratories were estimated by considering values of |Z| = 2 as acceptable, results in the range of 2 < |Z| < 3 as questionable, and, finally, values found to be |Z| = 3 as unacceptable. Summarizing, the Z-*score* was elaborated for each run only for Al, Cu and Zn. In Tables 7 the Z values accomplished by participants are listed.

From the evaluation of the outcome of the proficiency test, it can be stressed out that the Z-score overall trend was very satisfactory for most elements, except for few individual cases regarding Al. In fact, the quantification of this element in serum resulted to be particularly complicated. An example

Table 6. - The σ values assigned as a function of the concentration interval

	Concentration interval (µg l^{-1})	σ assigned (µg l^{-1})
Al	5.0 - 6.0	1.5
	6.5 - 7.5	2
	25 - 30	7
Cu	150 - 160	34
	350 - 450	80
	1,000 - 1,200	180
	1,400 - 1,600	240
	1,600 - 1,800	250
Zn	300 - 500	70
	700 - 800	135
	800 - 1000	150
	3,000 - 3,500	440

Table 7. - Z-score for Al, Cu and Zn

	I run	II run		III run		IV run	
Lab. no.	**Level 1**	**Level 1**	**Level 2**	**Level 1**	**Level 2**	**Level 1**	**Level 2**
Al							
1	0.0	-0.6	-0.2	-0.4	-1.4	-0.5	-
3	0.7	1.5	-0.7	0.1	0.4	-0.2	-1.8
4	0.4	0.8	0.3	0.0	0.9	1.7	3.0
6	0.4	1.6	0.2	1.9	2.9	-0.5	-0.8
15	1.9	0.7	0.3	-0.4	-1.1	3.2	0.2
20	-0.3	-1.8	-2.6	-0.1	1.7	4.5	7.0
23	-2.7	-0.5	0.4	0.0	0.1	1.8	1.1
25	3.3	2.3	1.9	1.0	-0.2	0.2	0.1
26	-2.4	-1.5	-2.4	0.6	1.0	-1.5	-2.3
27	-0.7	-	3.5	0.2	2.0	-0.3	-0.3
29	-0.8	0.0	-1.3	-0.3	-0.7	0.8	0.3
32	-	-2.2	-1.5	-0.7	-0.2	-0.2	-0.1
Cu							
1	0.0	0.1	0.1	-0.1	-1.2	-2.3	-2.2
2	3.6	0.4	0.0	0.7	0.0	0.1	0.2
3	0.3	0.2	0.1	0.0	0.1	-2.0	3.7
4	0.1	-0.2	-0.2	-0.2	-0.5	-0.3	-0.4
5	-2.0	-0.9	-0.4	-0.9	-4.0	-0.5	-0.7
7	-0.1	0.0	0.0	0.0	0.1	-0.3	-0.1
9	0.7	0.3	-0.1	1.5	0.1	0.6	0.4
11	0.0	0.1	0.0	-0.3	-0.3	0.8	0.7
15	1.2	0.3	0.2	-0.1	-0.4	-0.4	-0.3
23	1.2	0.8	1.0	0.4	0.1	0.0	-0.1
24	-3.9	-0.2	-0.3	0.6	0.3	0.4	0.3
27	0.5	0.0	0.0	0.0	0.0	0.5	0.5
29	-0.3	-0.2	-0.4	-0.1	-0.8	-0.2	0.1
Zn							
1	0.0	-0.6	-0.1	0.0	0.0	-1.7	-2.0
2	-0.8	-0.4	-0.5	0.8	0.4	0.0	-0.7
3	1.0	0.1	0.0	-1.0	-0.3	-0.4	0.7
4	0.4	-0.1	0.3	0.3	0.0	0.1	-0.2
7	0.2	0.0	0.0	-0.8	-1.1	-0.7	0.1
9	-	-0.4	0.0	-1.6	-0.3	0.2	-0.1
15	-0.3	0.0	0.8	-0.6	-0.4	-0.8	-0.6
23	-0.4	0.2	0.5	0.1	0.4	0.5	0.1
24	1.2	1.5	1.7	0.3	0.4	0.6	0.1
25	0.5	0.1	-0.4	-1.1	-0.2	0.0	-0.3
26	0.6	-0.2	-0.4	0.7	0.7	0.0	-0.2
27	0.2	-0.3	-0.3	0.0	-0.1	0.8	0.3
29	0.3	0.0	0.0	0.1	0.1	0.2	0.1
32	0.4	0.8	0.8	-0.1	1.1	-0.2	-1.3

-: not available.

was that of the laboratory no. 25, which in the first run achieved a value of Z = 3.3 (level 1), considered unacceptable. Subsequently, its performance during the trial has been improved till to reach acceptable values Z = 0.2 (IV run, level 1). The same was true for laboratory no. 32, that enhanced its performance, especially in the last run, from Z = -2.2 (II run, level 1) to Z = -0.2 (IV run, level 1). Instead, in the case of laboratory no. 20, the results were quite fluctuating with a strong worsening up to the fourth run. This confused trend could be probably ascribable to the unpredictable contamination of serum samples, during their manipulation, or to the insufficient attention payed to keeping the standard protocols of the analytical procedure. As regards Cu and Zn, only laboratory no. 24 gave results difficult to interpret and less satisfactory. Actually, for Cu, this laboratory obtained -3.9 in the first run (level 1), with a progressive improvement until the end. This attainment was probably due to the very dissimilar copper concentration between samples provided in the first run, at very low level, 159 ± 24 μg 1^{-1}, and the subsequent samples which had a range from 900 to 1,300 μg 1^{-1}, i.e., a common reference interval for humans. It is, thus, plausible that the laboratory no. 24 was unable to properly detect copper in serum at low level.

Conclusions

Participation in proficiency test is a benefit for all analytical laboratories wishing to control the quality of their experimental data. The results of these four phases of the interlaboratory trial on animal serum confirmed this assumption. In fact, most of the participants improved their performances, in terms of Z-score values, during the length of the exercise, but few exceptions for which further investigations are desirable.

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Annex 1. - *List of the laboratories participating in the proficiency test*

ALS 12, Biella; ALS 21, Casale Monferrato; Azienda Sanitaria Ospedaliera "San Giovanni Battista" Dip. di Patologia Clinica, Torino; Laboratorio di Analisi Chimico Cliniche, Presidio di S. Andrea, ASL 11, Vercelli; Diagnostica e Ricerca, S. Raffaele S.p.A., Milano; Azienda Ospedaliera "San Paolo Polo", Dip. Medicina del Lavoro, Milano; Istituti Clinici di Perfezionamento, Dip. di Medicina del Lavoro e Sicurezza, Lab. di Tossicologia Professionale, Milano; Ospedale Maggiore Policlinico, Dip. di Scienze Neurologiche, Milano; ASL, Lab. Analisi, Sondrio; ARPAV, Belluno; Azienda Ospedaliera, Verona; ARPAV, Servizio Laboratori, Verona; Ospedale "S. Bortolo", Laboratorio di Chimica Clinica, Vicenza; ARPAV, Friuli-Venezia Giulia, Gorizia; ASL 4 "Alto Vicentino", Thiene (Vicenza); Università di Perugina, Istituto Medicina del Lavoro, Perugia; ASL 3, Dip. di Patologia Clinica (DIPAC), Foligno - Perugina; ARPAL, Laboratorio, La Spezia; USL, Lab. di Tossicologia Unificato, Scandiano (Reggio Emilia); ASL 2, Lab. Sanità Pubblica, Area Vasta, Lucca; ASL 8, Ospedale S. Donato, UO di Biochimica Clinica, Arezzo; Azienda Sanitaria di Firenze, Lab. di Sanità Pubblica, Tossicologia Occupazionale e Ambientale, Firenze; ASL 1, Lab. di Tossicologia e Medicina del Lavoro, Pesaro; ALS 12, Laboratorio di Analisi, San Benedetto del Tronto (AP); ASL, Ancona; Policlinico Universitario "A. Gemelli", Lab. di Chimica Clinica, Roma; ALS 4, Dip. di Neuroscienze e Visione, Coppito (L'Aquila); ASL 4, Lab. di Chimica e Tossicologia, S. Anastasia (Napoli); ASL FG-3, Presidio Multizonale di Prevenzione, Settore Chimico, Ambientale e Tossicologico, Foggia; ASL 2, Servizio di Patologia Clinica, Lab. di Analisi, Casarano (Lecce); ASL 6, Lab. Igiene e Profilassi, Reparto Chimico, Sezione Analisi Metalli, Palermo; USL 8, Presidio Multizonale di Prevenzione, Area Chimica, Farmacologia, Ambientale, Cagliari.