An External Quality Assessment Scheme for Trace Elements in Biological Fluids

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The role for external quality assessment (EQA) schemes is illustrated by published results, which are probably inaccurate. The organisation and management of an EQA scheme for trace elements in biological fluids is described to show how the analytical performance of participating laboratories can be determined. The wider roles fulfilled by EQA schemes are discussed with examples of how the trace elements EQA scheme has been used to demonstrate the reliability of analytical data.

Keywords: *Quality assessment; trace elements; biological fluids*

Recent developments in analytical techniques make it possible to determine the concentrations of elements at low (ng ml^{-1}) levels using samples of only 5–50 µl.[1] However, measurements at these levels may be complicated by problems such as contamination during sample collection, storage or analysis, adsorption of analyte from sample on to the container and interferences associated with the complex nature of a biological matrix. The influence of improved control over contamination and other factors has been well illustrated by the work of Versieck and Cornelis.[2–4] Their various reports have demonstrated that reference values published from different laboratories are now showing some consistency at concentrations that are generally considerably lower than were stated less than ten years ago.

Despite recognition of these problems, results are still reported which, if examined critically, appear to be extremely unlikely. Among the anomolous results presented in one study[5] were the "normal" serum lithium concentrations of 0.48 and 0.78 mmol l^{-1}. These results are typical of those found in subjects receiving prophylactic lithium to treat depressive illness[6] and would not be found among many of the general population. A second series of results that is difficult to interpret is from work with neonates.[7] Although there are few other studies with which to compare, some of these data also appear to be quite unrealistic. The concentrations of gold in serum (mean ± SD) were 1.2 ± 0.45 µg g^{-1}, which are similar to those found in infants of mothers who received intramuscular injections of gold throughout pregnancy.[8] The concentrations of copper were equivalent to levels found in cases of copper toxicity and quite dissimilar from those reported by other workers.[9] Other unlikely results can be found in the published literature.

In recent years a different situation has developed with ultra-trace elements, where probable concentrations in blood are less than 10 µg l^{-1}. It is difficult to achieve reliable results for measurements made close to analytical detection limits, particularly where contamination is not easy to avoid. The establishment of reference ranges for healthy and other populations requires care and may be facilitated by inter-laboratory comparisons of results.[3,4]

External quality assessment (EQA) schemes should, if properly used by participants, prevent the publication of results of doubtful validity. Furthermore, participants with performance judged to be satisfactory by their EQA data can generally be confident about their results from samples having very low concentrations.[10,11] An EQA scheme for trace elements in biological fluids was established to provide objective evaluation of the analytical performance of laboratories that carry out these measurements. This paper describes the operation of the scheme and its application to biomedical analytical atomic spectrometry.

Experimental

The combination of analytes and sample types that are included in the scheme are given in Table 1. The approximate number of participants is 110 (September 1985) and these include laboratories from more than 15 countries throughout the world. Not all participants are included for every analyte - matrix combination.

The scheme is organised to give a succession of six-month cycles that commence in April and October each year. Every month, three specimens of each matrix are sent, as appropriate, to the participants. Each cycle includes, therefore, a total of 18 specimens. (The programme for aluminium in water and dialysis fluids is a little different with fewer samples, sent bimonthly, throughout an annual cycle.)

Samples of serum, blood and urine are prepared for distribution as described below. All laboratory-ware is cleansed by soaking overnight in 10% *V/V* HCl and rinsing thoroughly with de-ionised water. Batches of tubes used for storage and distribution of samples after preparation are screened and have been shown not to cause adventitious contamination to the contents.

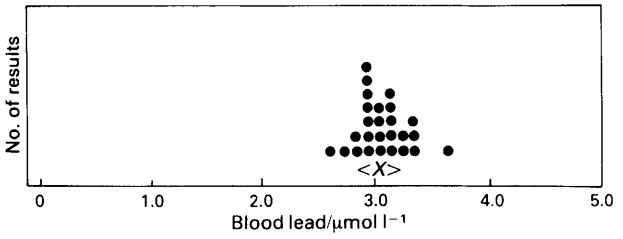
Serum. Chelex 100 ion-exchange resin (Bio Rad Laboratories Ltd.) is added to a single batch of sterile horse serum (Sera-Lab Ltd., Crawley Down, Sussex, UK) to reduce the endogenous concentrations of Cu and Zn to low to normal levels. Resin, 50 g l^{-1}, is added and the serum continuously stirred for 24 h. The suspension is centrifuged and the serum decanted into nine calibrated flasks. The concentration of Au, Cu and Zn are increased in eight of these pools by the addition of calculated amounts of standard solutions (BDH Spectrosol solutions) to the chelex-treated serum. A series of nine pools

Table 1. Analytes and samples included in the Trace Elements Quality Assessment Scheme. Figures in parentheses give the approximate number of participants for that analyte - sample combination (September 1985)

Serum	Blood	Urine	Water/dialysis fluid
Aluminium (50)	Cadmium (17)	Cadmium (12)	Aluminium (35)
Copper (65)	Lead (30)	Mercury (20)	
Gold (6)			
Selenium (12)			
Zinc (75)			

Table 2. Blood Pb results in samples prepared after erythrocyte lysis using saponin or ultrasonication. Results are mean ± SD of all values reported from two distributions

Sonicated samples			Saponified samples		
Pb added/ μmol l^{-1}	Mean ± SD/ μmol l^{-1}	Recovery, %	Pb added/ μmol l^{-1}	Mean ± SD/ μmol l^{-1}	Recovery, %
0	0.28 ± 0.047	—	0	0.27 ± 0.059	—
0.8	1.12 ± 0.059	106	—	—	—
1.0	1.27 ± 0.055	100	1.0	1.41 ± 0.090	114
1.6	1.96 ± 0.122	106	2.0	2.35 ± 0.104	104
2.8	3.04 ± 0.106	99	3.2	3.47 ± 0.160	100



Sample number 72
Mean . . . . . 3.07 μmol l^{-1}
Standard deviation . . . . . 0.21 μmol l^{-1}
Coefficient of variation 6.73%
Range of results . . . . . 2.67–3.62 μmol l^{-1}
Number of values reported 26

Trace Element Quality Control: Blood Lead Results

2	3.62	4	2.90	5	3.15	8	3.03	10	2.95
15	2.93	17	2.92	25	2.84	26	3.04	31	3.15
35	2.98	36	2.93	38	2.67	23	3.35	47	3.28
50	2.94	64	3.19	67	3.12	70	3.00	72	2.80
78	3.06	79	3.23	84	3.34	89	3.11	93	3.33
103	2.93								

Fig. 1. A page from a monthly report for lead in blood showing the histogram, calculations and tabulation of results alongside the appropriate laboratory code number

supplemented with Al and Se are similarly prepared but without the preliminary chelex treatment. The samples are mixed and dispensed into labelled tubes (2.0-ml trace-element tubes, Tek Lab, Sacriston, Durham, UK).

Blood. Human blood is collected from a volunteer and contains dipotassium ethylenediaminetetraacetic acid, 2 mg ml^{-1}, to prevent coagulation. (Samples are screened for Australia Antigen and HTLV III antibodies before any further preparation is undertaken.) A comparison of ultrasonic energy and the use of saponin to produce lysis of erythrocytes was made where two portions of blood from the same donor were subjected to both techniques and thereafter treated identically. No difference between the results reported by participants were found with respect to precision and recovery (Table 2), and erythrocyte lysis is routinely accomplished by the addition of saponin to the blood. The haemolysed blood is transferred into calibrated flasks and the concentrations of Cd and Pb augmented as for elements in serum. The blood is mixed and then dispensed into labelled tubes.

Urine. Human urine collected from volunteers who have been screened and found not to carry Australia Antigen or HTLV III antibodies is placed into calibrated flasks and supplemented with Cd and Hg. Samples are mixed and dispensed into labelled tubes.

All samples of serum, blood and urine are subjected to gamma-irradiation (minimum dose 24 kilogray) to destroy any bacterial contamination that may have occurred during preparation. These specimens are stored at −20 °C until despatched. To determine that samples have been properly prepared and that there has been no contamination or other cause for between-tube variation, the elements included in the EQA scheme are measured in this laboratory in at least ten specimens from each batch prior to despatch.

Preparation of the serum samples is carried out at the beginning of a six-month cycle and each of the nine pools is distributed for analysis on two separate occasions to allow determination of between-batch precision. Blood and urine samples are prepared monthly. Accurate supplementation of the pools with the analytes permits subsequent calculations of recoveries of the amounts added.

Analysis of Results

Participants are asked to return their results within one month of the date of despatch. Results received before that time are used to prepare reports for each sample - matrix - analyte. An initial calculation of the mean and standard deviation (SD) is made and any result outside the range "mean ± 3SD" is omitted from the report. This monthly report gives the consensus mean, SD and coefficient of variation, a histogram of distribution and a tabulation of results (Fig. 1).

At the end of a 6-month cycle an end of term report is prepared, which summarises the 18 results for each sample - matrix - analyte combination and presents an assessment of analytical performance based upon parameters of: proximity to consensus mean ($\bar{X} - X$), difference between the results for samples analysed on two occasions ($X_1 - X_2$) and recovery of added analyte (%R). For each of these parameters we have adopted the procedure developed by Yeoman (described by Vahter[10]) for the establishment of targets or markers of satisfactory performance. These targets are based on what is necessary for clinical purposes and what can be achieved with available analytical techniques. A graphical example of this approach is shown for serum zinc in Fig. 2. Limits are selected at a high and a low level (±1.5 μmol l^{-1} at 20.0 μmol l^{-1} and ±1.0 μmol l^{-1} at 4.0 μmol l^{-1}, for Zn in serum) and these are joined to give targets that cover all concentrations. A second series of targets, closer to the mean, is drawn from limits that are half of those of the first set. In this way inner (A) and outer (A + B + C) target zones are established (Fig. 2). The limits selected for each analyte for the preparation of these zones are given in Table 3. Those shown for Pb in blood were first used by Yeoman,[10] others were suggested by us. Fig. 3 presents results for proximity to the consensus mean ($\bar{X} - X$) for three laboratories. These examples demonstrate: accurate performance, laboratory (*a*), where all results are close to the mean and are within the targets; good performance but with an obvious negative bias, laboratory (*b*); poor accuracy where few results are within the target ranges, laboratory (*c*). Fig. 4 shows how a similar display can be used for the assessment of precision (X_1–X_2). This example is also for Zn in serum.

Whitehead[12] recommends that EQA schemes should include a calculation of a performance score. We have used the scoring system elaborated by Yeoman for the Supra-regional Assay Service and adopted for the CEC Blood Lead

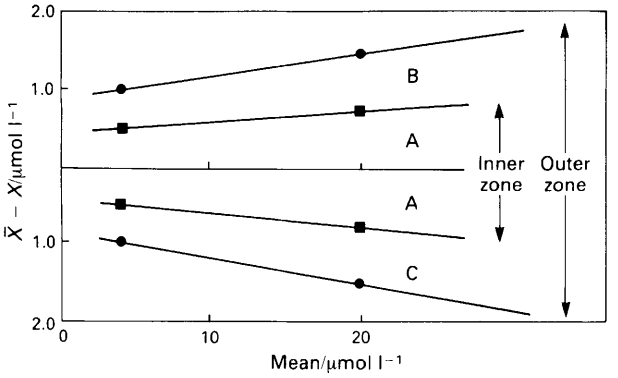


Fig. 2. Preparation of target zones for the assessment of performance. The example here is for Zn in serum: ●, points used to delineate the outer zone and ■, points used to delineate the inner zone

Table 3. Limits used for each matrix - analyte combination for the preparation of graphs to show accuracy and precision, *e.g.*, Figs. 2–4

Assay	Inner limits	Outer limits
Serum Al/μmol l^{-1}	0.2 at 1.0 0.4 at 4.0	0.4 at 1.0 0.8 at 4.0
Serum Au/μmol l^{-1}	0.25 at 4.0 0.60 at 16.0	0.5 at 4.0 1.2 at 16.0
Serum Cu, Zn/μmol l^{-1}	0.50 at 4.0 0.75 at 20.0	1.0 at 4.0 1.5 at 20.0
Serum Se/μmol l^{-1}	0.06 at 0.75 0.10 at 2.00	0.12 at 0.75 0.20 at 2.00
Blood Cd/nmol l^{-1}	4 at 50 7.5 at 200	8 at 50 15 at 200
Blood Pb/μmol l^{-1}	0.07 at 0.48 0.12 at 2.90	0.14 at 0.48 0.24 at 2.90
Urine Cd/nmol l^{-1}	5 at 50 12 at 300	10 at 50 24 at 300
Urine Hg/nmol l^{-1}	5 at 50 15 at 500	10 at 50 30 at 500

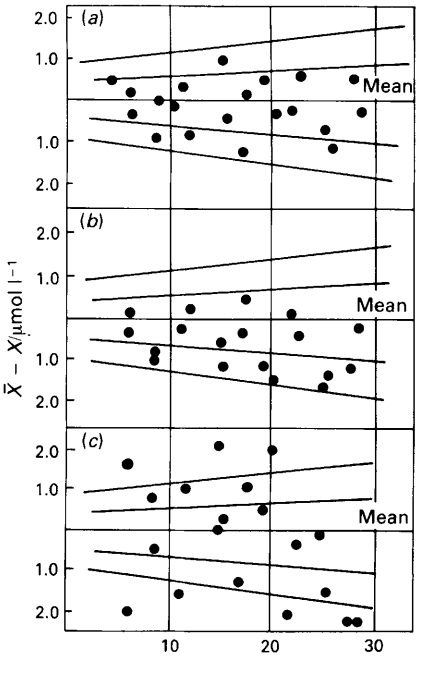


Fig. 3. Graphical display of targets used to assess proximity to consensus mean for zinc in serum. Laboratory (*a*) = satisfactory performance; laboratory (*b*) = good accuracy but a low bias; and laboratory (*c*) = poor accuracy

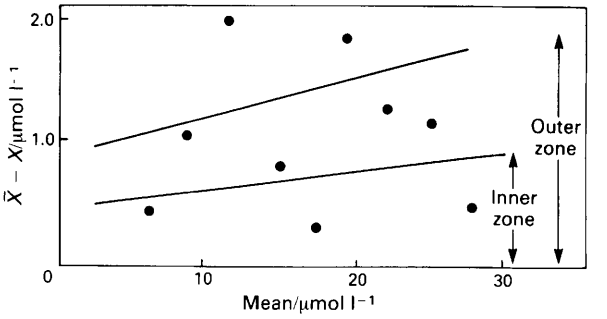


Fig. 4. Graphical display of assessment of between-batch precision, samples analysed on two separate occasions. This example is for Zn in serum

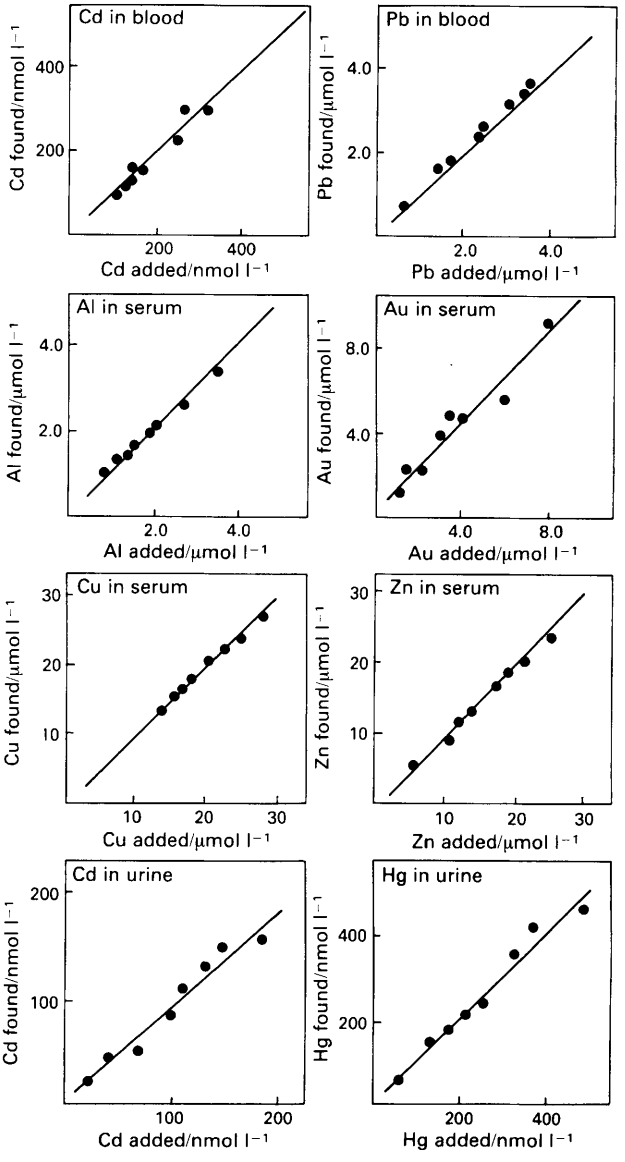


Fig. 5. Comparisons between consensus mean values (endogenous levels) and spike levels for Cd and Pb in blood, Al, Au, Cu and Zn in serum and Cd and Hg in urine. Data are from samples distributed between April and September 1985. Regression equations and correlation coefficients are as follows. Blood, Cd $Y = -9.847 + 0.9941 \times X$, $r = 0.9851$; Pb $Y = 0.037 + 0.9514 \times X$, $r = 0.9974$. Serum, Al $Y = 0.049 + 1.0197 \times X$, $r = 0.991$; Au $Y = 0.576 + 1.0054 \times X$, $r = 0.9767$; Cu $Y = 0.461 + 0.9637 \times X$, $r = 0.998$; Zn $Y = -1.149 + 1.0235 \times X$, $r = 0.9996$. Urine, Cd $Y = 1.725 + 0.9158 \times X$, $r = 0.9846$; Hg $Y = 5.487 + 0.9957 \times X$, $r = 0.9897$

Table 4. Results for Al, Cu and Zn obtained on two samples of serum: (*a*) within 1 month of preparation and (*b*) 6 months later after storage at −20 °C

	Aluminium			Copper			Zinc		
	No. of results	Mean ± SD/μmol l^{-1}	CV, %	No. of results	Mean ± SD/μmol l^{-1}	CV, %	No. of results	Mean ± SD/μmol l^{-1}	CV, %
(*a*)	31	1.27* ± 0.38	29.9	62	17.76 ± 2.29	12.9	66	13.04 ± 1.48	11.3
(*b*)	30	1.18* ± 0.20	16.9	53	17.81 ± 1.71	9.6	59	12.84 ± 1.26	9.8
(*a*)	30	3.46 ± 0.61	7.6	59	23.54 ± 2.45	10.4	64	2.70 ± 0.99	36.7
(*b*)	29	3.44 ± 0.51	14.8	55	23.98 ± 2.63	11.0	58	2.87 ± 1.16	40.4

* Significant difference $p = <0.05$.

Table 5. Mean inter-laboratory coefficients of variation (%) at the concentrations shown, from 1975 to 1985

	Serum			Blood			Urine	
Date	Al 2–3 μmol l^{-1}	Au 5–10 μmol l^{-1}	Cu 23–27 μmol l^{-1}	Zn 14–17 μmol l^{-1}	Cd 80–120 nmol l^{-1}	Pb 2–3 μmol l^{-1}	Cd 50–100 nmol l^{-1}	Hg 250–300 nmol l^{-1}
1975	—	—	—	—	—	19.2	—	—
1978	—	—	8.9	17.5	—	8.9	—	—
1981	41.6	14.0	9.5	12.5	19.8	7.4	31.9	27.8
1982	42.4	16.9	11.2	12.4	24.1	6.6	18.7	17.9
1983	32.0	11.1	8.3	14.1	22.2	6.6	24.8	22.0
1984	16.4	19.1	8.7	9.4	18.9	6.8	30.3	20.0
1985	16.1	23.0	9.0	10.6	14.9	5.5	17.5	23.0

Survey.[13] From the 18 results returned by a laboratory for, for example, Zn in serum, during a cycle, a performance score is calculated from the sum of: the percentage of results within the inner zone of the $\bar{X} - X$ graph, the percentage of results within the outer zone of the $\bar{X} - X$ graph, the percentage of results within the inner zone of the X_1–X_2 graph, the percentage of results within the outer zone of the X_1–X_2 graph and the percentage of recoveries within the range 90–110%.

A perfect score would be 500, we suggest that good performance is indicated by a score of 360. As analytical techniques improve this target score can be increased or made more difficult to achieve by refinement of the zones shown in Fig. 2.

Results and Discussion

An external quality assessment scheme will be of use only if certain fundamental conditions are fulfilled. These include (*i*) the distribution of specimens that are homogenous both internally and from tube to tube, (*ii*) samples that are stable throughout their anticipated period of use and (*iii*) the performance of valid computations with results. Careful preparation and preliminary analysis of a number of samples from every batch has ensured that the specimens distributed for the trace element EQA scheme are homogeneous. Specimens of sera from the same pools are analysed twice with intervals of up to six months between the two distributions. As shown in Table 4 there is no evidence of instability or deterioration of the samples and with the exception of one set for Al (where the second set of data are actually superior), results for all elements show no significant changes over this length of time. Other specimens included in the scheme are not stored for long periods. Some aspects of assessment of performance used in this trace element EQA scheme are calculated by reference to the consensus mean and this value may not necessarily be a good representation of the true concentration, particularly if there are only a small number of results. Georges,[14] however, has demonstrated that the consensus mean is generally adequate even when there are few participants. Furthermore, good accuracy and the validity of the consensus mean will be demonstrated if this value and the spike level are identical. Fig. 5 shows data from the period April to September 1985 and indicates good agreement for almost all of the elements. The inference from these results is that the consensus mean is valid except for Au in serum. This last analysis is carried out by no more than five participants and results require very cautious interpretation. Mean values from results obtained using similar methods have not been routinely calculated but the code numbers of laboratories using particular methods are made available to participants so that they can determine group-method means for their own use.

The International Federation of Clinical Chemistry (IFCC) expert panel on Nomenclature and Principles of Quality Control, described a series of objectives that external quality control procedures should seek to achieve.[15] The work and results of the Guildford trace elements EQA scheme has been applied to all of these objectives.

Investigation of Analytical Methods in Use

The programmes for Cu and Zn in serum have sufficient numbers of participants for results to be used to prepare a comparison of methods used for sample preparation.[16] It was shown that protein precipitation with trichloroacetic acid gave higher results (mean difference = +10.9%) than when sera were diluted with water or butanol and that the use of ETA-AAS produced low results for copper (mean difference = −3.9%).

Assessment of New Methods and Verification of Experimental Results

New methods for the measurement of trace elements are continually under development. To show that a novel procedure gives satisfactory results, experiments to demonstrate acceptable accuracy and precision should be carried out. The analysis of EQA samples with results compared against the consensus or group-method means, provides an independent

check of the performance of the new procedure. Results from investigations that have involved the trace element EQA scheme have been presented in many publications, for example in references 17–19. Furthermore, the analytical aspects of clinical investigations have been demonstrated to be reliable by the acceptable performance of the laboratory in the EQA scheme.[20]

Presentation of "State of the Art" and Stimulation for Improved Performance

The survey of performance prepared at six-month intervals identifies groups of laboratories whose scores are consistently high. It is evident that analytical performance can be maintained at standards appropriate or superior to those demanded for effective use of the results by clinicians and other workers. These standards of high performance are indicative of the best that can be achieved for the determination of trace elements in biological samples with the type of equipment generally available to hospital and other laboratories and represent the "state of the art" for such analytical procedures. Laboratories that fail to attain suitable (not necessarily the best) levels of performance should examine their procedures, methods, instrumentation etc. to determine which factors should be changed or improved in order that their analytical data match that which has been shown to be possible.

A good example of poor performance revealed by the trace elements EQA scheme is afforded by the determination of Al in serum. This measurement is important in the management of patients with chronic renal disease. These subjects are likely to absorb Al from contaminated dialysis fluids or from orally administered hypophosphataemic agents, with consequent toxicity. When the unsatisfactory analytical situation was realised, considerable efforts to bring about an improvement were made. The six-month reports have shown that the number of laboratories that achieve the target score for the determination of Al in serum has increased in the last three years and that the inter-laboratory variation between results has gradually declined.[21] Many factors are involved in this trend but regular monitoring of performance with demonstration of the variation between results from laboratories has been one such factor.[19] Changes in inter-laboratory variation during 1975–1985, which reflect the improved performance for the measurement of trace elements in biological fluids, are given in Table 5. The results show that for certain elements there has been little change in the spread of results between laboratories but that for analyses that have received special attention for statutory or other purposes, such as Pb in blood and Al in serum, the situation has gradually improved. Additional data for these latter determinations have been given elsewhere.[21]

Education

Management of any EQA scheme provides opportunities for the collection of large amounts of information and data pertinent to the analyses included in the scheme. This can be made available to participants by direct communication, in special reports or publications or by the provision of scientific meetings. Those responsible for the organisation of the trace elements EQA scheme are frequently asked by participants for information and advice to help them to solve particular problems. Meetings have also been organised, including an international conference on the role of aluminium and other trace elements in renal disease.

While these aspects of the EQA scheme are important in the work of laboratories concerned with measurements of trace elements the most important function is to supplement any internal quality control procedures that may be in operation. The principles and practices described here were elaborated for the assessment of analytical performance with respect to trace element determinations in biological samples. They are also relevant to different applications and other techniques, in addition to atomic absorption spectrometry.

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