

## Reference Materials and Analytical Standards to Stimulate Improved Laboratory Performance: Experience from the External Quality Assessment Scheme for Trace Elements in Biological Samples

Andrew Taylor

Trace Element Laboratory, Robens Institute, University of Surrey, Guildford GU2 5XH, England

**Abstract.** The performance of a large number of laboratories measuring trace elements in biological fluids has been monitored over many years by examination of their results in the Guildford external quality assessment scheme. Specific experiences of the UK trace elements reference laboratories have been used to stimulate improvements in performance of other participants in the scheme. The key features of these initiatives were: specially prepared reference materials, used as internal quality control specimens within a common procedure, contributed to accuracy control; proposed standards of satisfactory and unacceptable analytical performance associated with a new system for scoring; regular non-threatening open discussion of performance with interested colleagues. The impact of these features is illustrated with reference to measurements of Al and Zn in serum and Pb in whole blood.

**Key words:** reference materials, internal quality control, external quality assessment, analytical standards, peer review.

Analysts concerned with measurement of trace elements in biological specimens are faced with a number of requirements not usually encountered by their colleagues in other areas of laboratory medicine. The most obvious of these is the necessity to avoid adventitious contamination. With concentrations in the nanogram per gram range and sample sizes of a few microlitres or milligrams, even the slightest trace of contamination will invalidate a measurement. Therefore, steps such as collection, storage and preparation for analysis have to be carried out with a degree of attention which would appear absurd to someone measuring glucose or haemoglobin. This constraint is widely recognized and documented [1–3].

Further requirements that may be noted refer specifically to the analytical procedure. The low concentration and small sample size demand that techniques with exquisite sensitivity are employed. Even with such techniques the measurement

will often be close to the detection limit where the inherent imprecision rapidly increases and the entire procedure must be conducted in such a way so as to favour good reproducibility. Important clinical decisions may be made on the basis of a result; a child with a blood Pb level of 9  $\mu\text{g}/\text{dl}$  is said to be well whereas a result of 10  $\mu\text{g}/\text{dl}$  is claimed to represent Pb poisoning [4, 5]. Methods must also, therefore, have proven accuracy.

These requirements – sensitivity, reproducibility and accuracy – are facilitated by features within the laboratory such as good modern equipment, dependable methods, experienced and competent staff. However, special approaches are also needed so as to make best use of these features and to demonstrate their effectiveness. Incorporation of such approaches into the organization of trace elements laboratories to provide a positive influence for good analytical performance will be discussed in the following sections.

### Reference Materials and Internal Quality Control

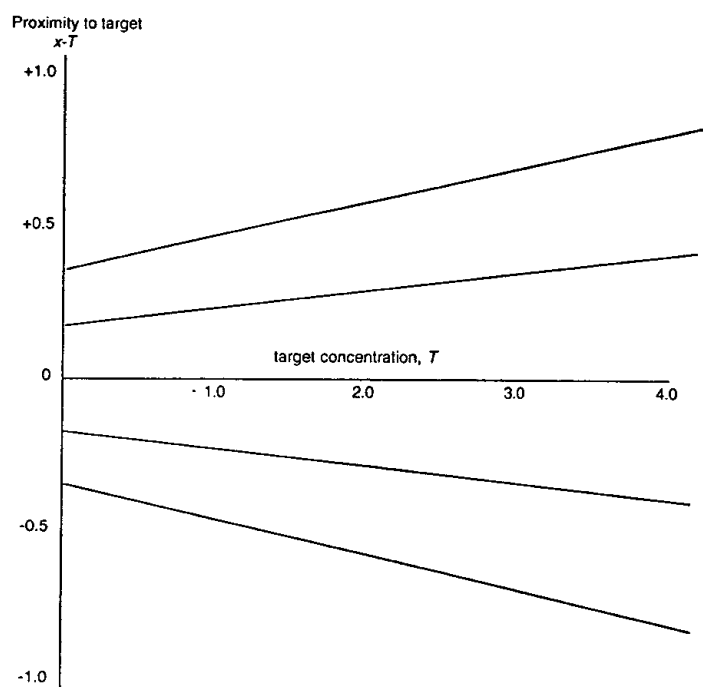
Reference materials (RMs) are used for a number of purposes [6]. Certified RMs (CRMs), with carefully defined concentrations, are valuable for demonstration of the accuracy of an analytical procedure or for calibration of an instrument or a method. External quality assessment schemes (EQASs) involve the distribution and analysis of RMs by all participants in the programme and comparison of the results so that accuracy and other parameters can be independently monitored [7]. Features of EQASs are discussed in the next section. Internal quality control (IQC) procedures usually monitor analytical precision and most rely on the use of RMs.

The trace element reference laboratories in the United Kingdom have slightly modified their use of RMs for IQC in such a way as to produce improved precision and accuracy for the measurement of Pb in blood [8, 9]. The principles have since been applied to analyses for Al, Cu and Zn in serum. To achieve the twin objectives of good precision and accuracy it has been necessary to prepare, or to commission the preparation of, appropriate RMs. A set of RMs is prepared from a single specimen divided into the required number (usually two or three) of sub-samples. One sub-sample receives no further treatment, while the endogenous concentration of analyte in others is carefully enhanced by a predetermined amount. The sub-samples are mixed and dispensed into metal-free tubes for storage.

These materials have good long-term stability. The concentrations of Pb in a number of RMs stored for up to 7 years are shown in Table 1 and no significant changes were detected. The target concentrations assigned to these materials were derived from the results obtained by reference laboratories during several analytical batches over six to eight weeks. In addition, samples were measured at specialist laboratories using other analytical techniques and the measured concentrations closely match the expected values based on the amounts added during preparation [8]. It is concluded, that assigned concentrations are very close to the 'true' values. Working limits around these target concentrations were derived from the inner zone of the charts used for performance assessments in the Guildford Trace Elements EQAS. The chart for the serum Al scheme is shown in Fig. 1 as an example [7]. The key feature of these charts is an increased range, when expressed as a percentage of the sample concentration, at concentrations close to the analytical detection limit.

**Table 1.** Stability of reference materials for Pb in blood. Concentrations ( $\mu\text{mol/l}$ ) were accessed by the UK supra-regional assay service reference laboratories after storage at  $-20^\circ\text{C}$  for up to 7 years

Material	1984	1985	1987	1992
A	0.70	0.67	—	—
B	1.82	1.79	1.78	—
C	3.20	3.17	3.22	—
X		0.55	0.55	0.55
Y		1.67	1.68	1.66
Z		3.32	3.31	3.29
P			0.46	0.46
Q			1.57	1.54
R			2.99	2.99



**Fig. 1.** Zonal chart used for assigning working limits to an IQC sample. The target concentration ( $\mu\text{mol/l}$ ) is determined as described in the text and plotted on the horizontal line. The corresponding concentration at the line between the inner and outer zones defines the limits to be used with that sample. The chart is also used in calculation of performance in the EQAS. The procedure for drawing the zones is given in ref. [7]

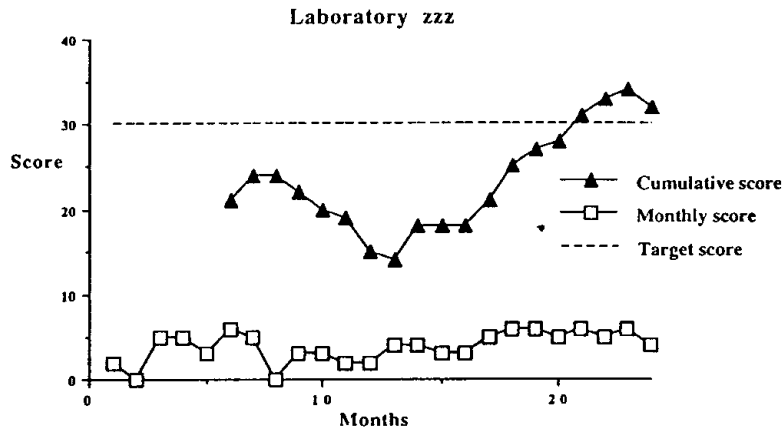


Fig. 2. Performance for one participant in the serum AI EQAS, monitored for 12 months before and after the introduction of a common IQC procedure

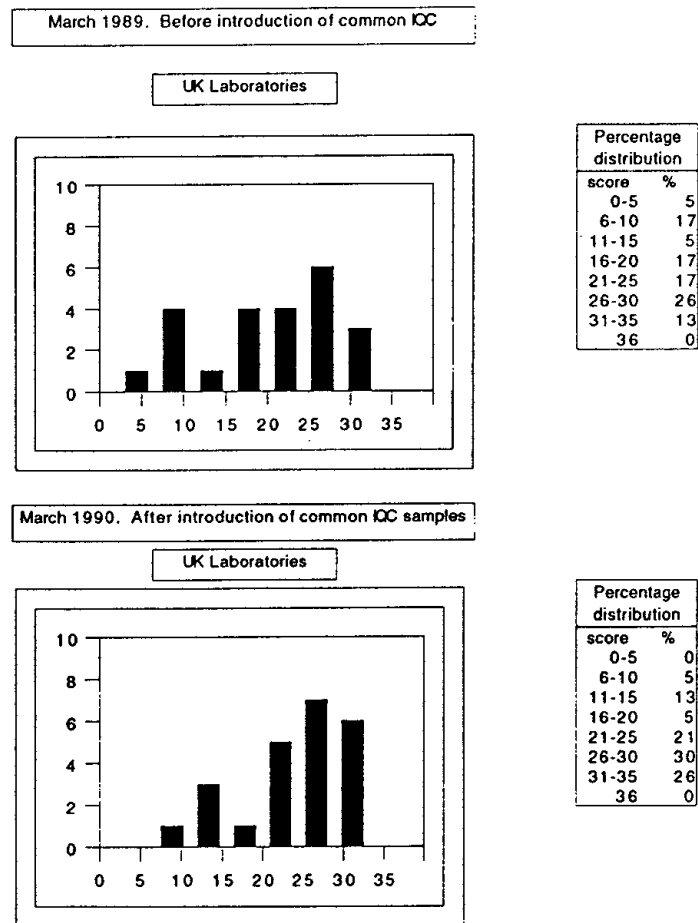


Fig. 3. The distribution of cumulative scores achieved by UK participants in the serum AI EQAS before, and twelve months after, commencement of the common IQC procedure

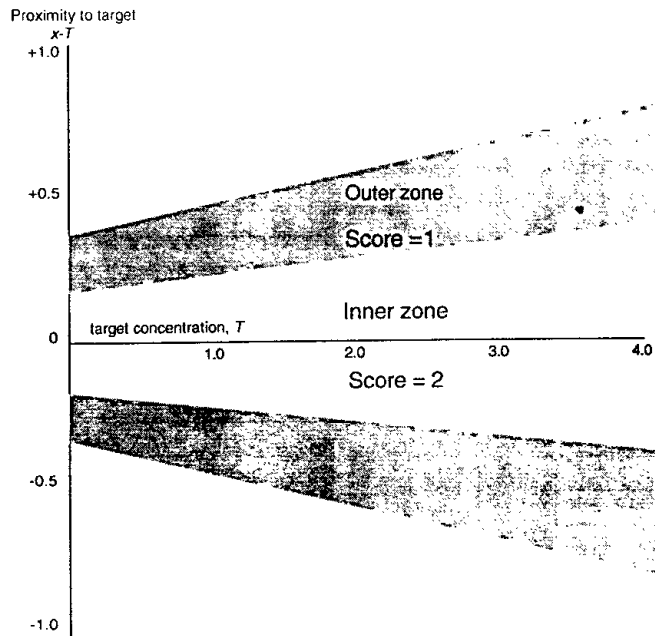


Fig. 4. Zonal chart used for scoring performance within the EQAS for serum Al. Proximity of a result to the target concentration ( $\mu\text{mol/l}$ ) is plotted and scores 2, 1 or 0 if within the inner zone, outer zone or outside the shaded limits, respectively

A common IQC, with all collaborators using these RMs in the same way, should lead to improved comparability of analytical data.

An example to illustrate this concept is from the United Kingdom, where it was decided by participants in the serum Al EQAS to adopt the practice of common IQC in April 1989. Figure 2 shows the performance of one laboratory in the EQAS scheme for twelve months before and after this initiative. This particular procedure for assessment of performance was not developed until 1990 and, therefore, Fig. 2 gives a retrospective display which was not seen by the participants during the period reviewed. Two scores are given: the *monthly score* refers to the set of 3 specimens analyzed during the previous month, while the *cumulative score* is the sum of the previous six monthly scores. In the system employed for this scheme a higher score represents better performance. It is evident that unsatisfactory results were reported during the first phase, but following the introduction of the common IQC strategy a significant improvement in performance was produced. Similar improvements were also seen for many of the UK laboratories. Figure 3 presents the distribution of cumulative scores achieved by UK participants before, and twelve months after, commencement of the common IQC procedure. The 'shift-to-the-right' is produced by a larger proportion of participants with higher scores. The concept for common IQC was developed for blood Pb analyses some years earlier by Yeoman and implemented by the UK reference laboratories [10]. It was subsequently adopted for European blood Pb surveys and by other group of laboratories.

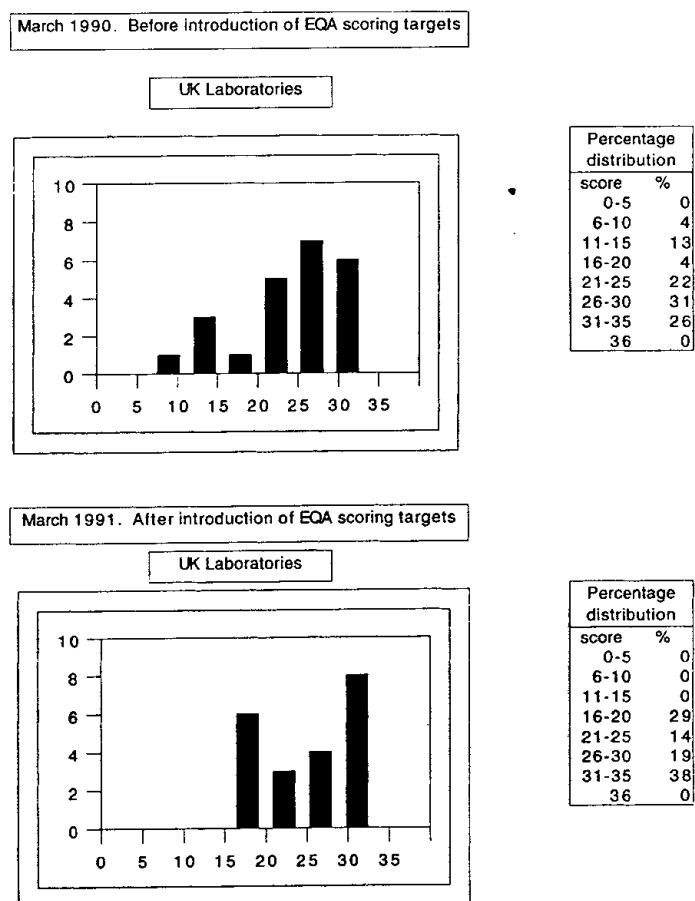


Fig. 5. The distribution of cumulative scores achieved by UK participants in the serum AI EQAS before, and twelve months after, introduction of analytical standards

The concept has been successfully extended to larger number of laboratories and for a wider range of analytes.

#### Analytical Standards and External Quality Assessment

As shown in Figs. 2 and 3 the common IQC procedure can give significant improvements in laboratory performance as measured in the EQAS. However, some laboratories in the UK were unable to reach as high a level of performance for measurement of AI in serum as other participants. This led to a further initiative in 1990. Prior to this date performance assessments were made every six months and were inevitably somewhat out of date to be of immediate benefit to participants [7]. A revised system was introduced in 1990, with the monthly and cumulative scores referred to above, and based on the proximity of a result to the target concentration ( $x - T$ ). For each specimen assayed the proximity of a participant's result to the target concentration is plotted onto a zonal chart, as shown in Fig. 4.

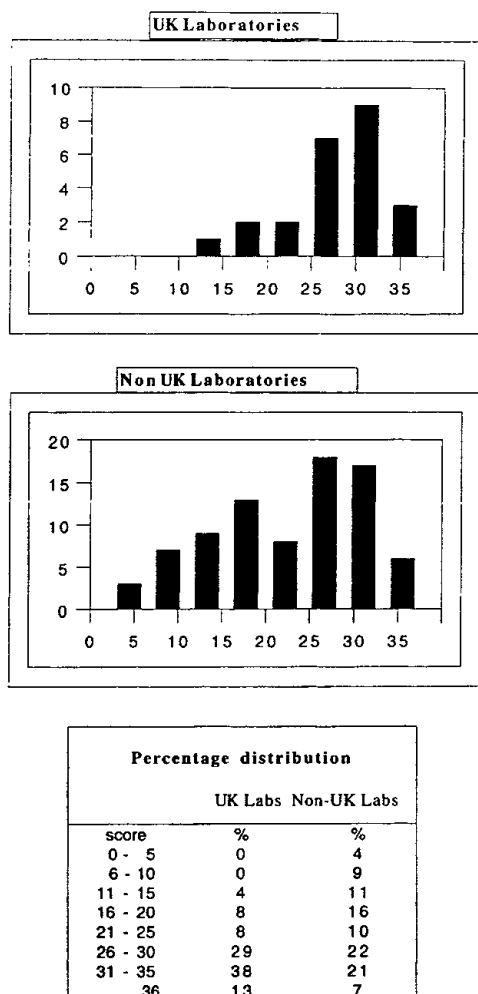


Fig. 6. The distribution of cumulative scores achieved by UK and non-UK participants in the serum AI EQAS in July 1994

A point in the inner zone gains a score of 2, a point in the outer zone scores 1, while for anything outside the limits the score is 0. Each month there are three samples so that a good performance is indicated by a *monthly score* of 6. As stated above, the *cumulative score* is the sum of the most recent 6 monthly scores and will have a maximum of 36. It was suggested by the scheme organisers that for UK laboratories there should also be standards associated with these scores. A proposal that a cumulative score of 30 or more is indicative of acceptable performance and that a score of below 25 is unacceptable was agreed. It was also agreed that if a participant had a cumulative score below 25 in a sequence of three out of four months, this unacceptable performance would be reported to the UK Advisory Panel for Quality Assurance. (Although the UK does not have a mechanism for licencing or certification of laboratories, reports to the Advisory Panel are seen to be unfavourable and are taken into consideration when applying for accreditation).

Introduction of these standards, in association with the revised scoring procedure, brought about further improvements in performance. Figure 5 gives the

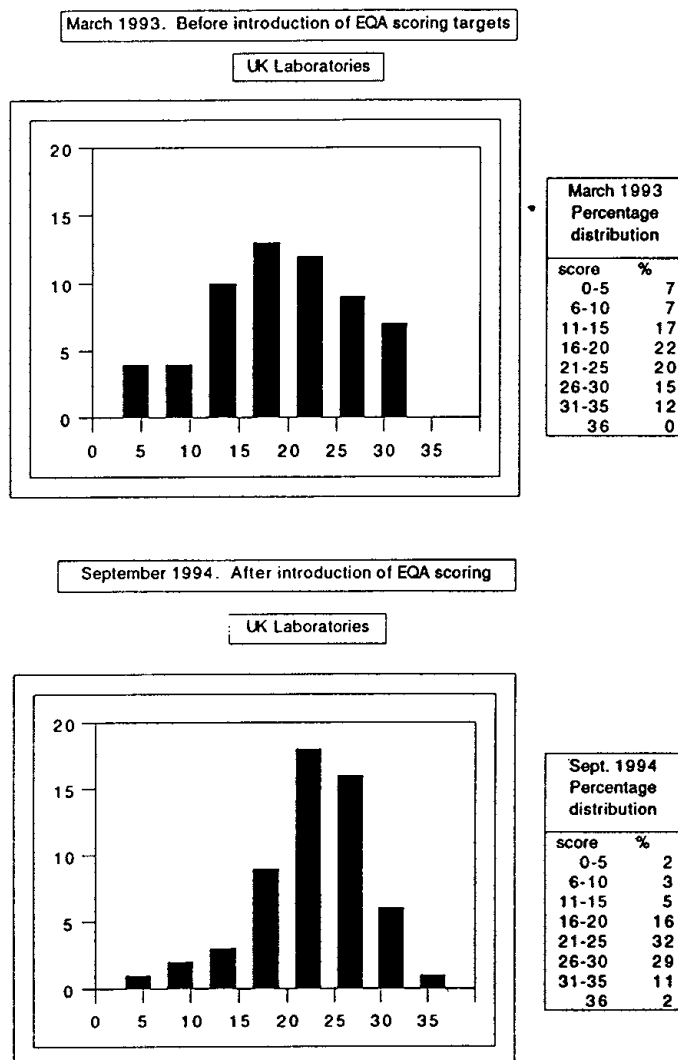


Fig. 7. The distribution of cumulative scores achieved by UK participants in the serum Zn EQAS before, and twelve months after, introduction of analytical standards

distribution of cumulative scores in March 1990, immediately before the implementation of these standards, and one year later. This improvement has been maintained and a further 'shift-to-the-right' is seen in the data for July 1994 (Fig. 6). This figure also shows that performance within the UK is superior to that of non-UK laboratories (as a group), who were not subject to the same demands. Analytical performance standards have similarly been presented for the measurement of Cu and Zn in serum. As a consequence there has been a consistent, overall improvement in performance, as shown in Fig. 7 for serum Zn with the same 'shift-to-the-right' as seen for Al. In addition, certain laboratories that were unable to meet these standards have voluntarily withdrawn an unsatisfactory analysis from their repertoire of tests.



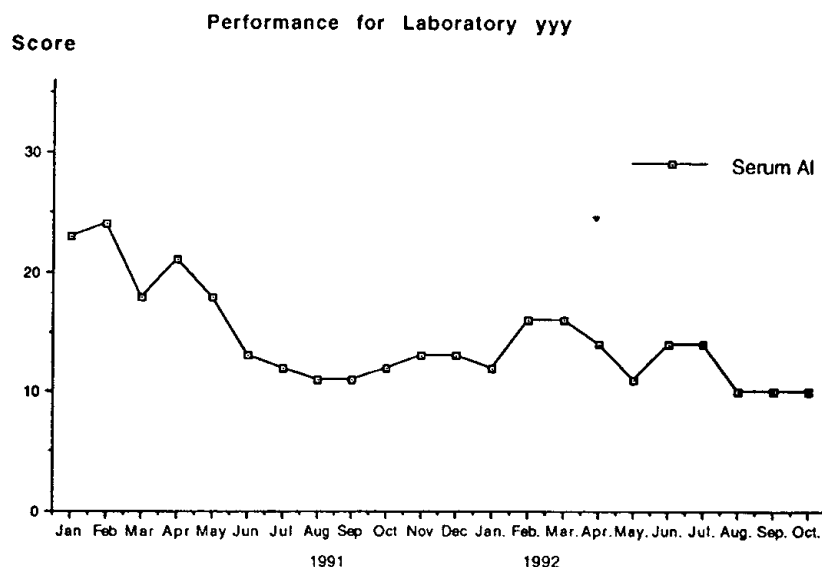


Fig. 8. Long term performance for one participant in the serum AI EQAS, where data were kept secret and problems were not shared with other members of the laboratory

### Peer Review

In addition to pioneering the common approach to IQC and helping to establish the analytical performance standards, the trace element reference laboratories in the United Kingdom collaborate to develop and improve trace element laboratory medicine. These activities are maintained by regular meetings which include an open review of quality assessment data together with mutual support if there are instrumental or methodological problems.

Opportunities for discussion of performance between laboratories in this way are unusual and are not encouraged by the evolution of attitudes of market competition and commercialism. Nonetheless, the positive aspects of peer review are evident and Table 2 shows the excellent between-laboratory comparability for Pb in blood among the UK trace element reference laboratories, even at very low concentrations. This has been maintained for almost twenty years and there are many further examples of the accuracy and precision achieved by these laboratories [9, 11]. However, in addition to improvements in analytical quality the Trace Elements EQAS reveals situations of declining performance. Figure 8 is an example from a laboratory with inconsistent and generally poor results in the serum AI scheme. Eventually it was disclosed that just one individual was responsible for the work, that EQA reports were not seen by anyone else within the laboratory and that the unsatisfactory performance was not shared with his colleagues. Not only was there any discussion with others who might have been able to help, but there was not even a review within the department. Such secrecy is not common, but organizers of EQASs are aware of instances where staffing and managerial constraints actively inhibit opportunities for performance reviews.

**Table 2.** Measurement of Pb in blood. Between-laboratory precision at concentrations below 0.7245  $\mu\text{mol/l}$  (15  $\mu\text{g/dl}$ ) in UK reference laboratories

Number sample	$\mu\text{mol/l}$ Concentration	CV (%)
802	0.17871	21.1
809	0.17871	29.0
796	0.27048	24.1
781	0.28014	18.3
773	0.31878	15.7
823	0.32844	13.0
827	0.34776	8.7
763	0.38157	16.0
775	0.60858	15.7
806	0.60858	9.8
819	0.6279	8.1
769	0.65205	8.0
738	0.66171	6.6
797	0.68103	9.1
795	0.69069	7.6
826	0.69069	4.8

### Conclusions

The high quality of analytical performance sustained over many years by the trace element reference laboratories is not unique to this small British group [9, 11]. Other laboratories are capable of reporting excellent data investigations clearly indicate that this is associated to a specialist interest within the laboratory or department [11]. It is now apparent that results can be improved in general clinical laboratories and that carefully planned IQC, relevant analytical standards and regular non-threatening open discussion of performance are the tools to stimulate improvements in performance.

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