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The Guildford trace elements external quality assessment scheme

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Summary. - An international external quality assessment scheme for trace elements in biological fluids is described. Programmes for aluminium, copper, zinc, selenium, gold, lead, cadmium and mercury in serum, blood, urine, dialysis fluids and water are included with others under development. Preparation of reports is described and assessments of performance based on proximity to assigned values, differences in results between duplicate measurements and recovery of added analyte are given. Performance standards have been introduced to stimulate improvements in the quality of results.

Key words: trace elements, external quality assessment, biological fluids, performance standards, Great Britain.

Riassunto *(Il programma di valutazione esterna della qualità di Guildford, UK, per gli elementi in traccia)*. - Viene descritto un programma internazionale di valutazione esterna di qualità per gli elementi in traccia in fluidi biologici. Attualmente il programma comprende schemi per la determinazione di alluminio, rame, zinco, selenio, oro, piombo, cadmio e mercurio nel siero, sangue, urine, fluidi di dialisi e acqua; altri schemi sono allo studio. Viene descritta la preparazione dei rapporti e la valutazione delle prestazioni in base allo scarto dal valore atteso, le differenze tra misure in duplicato e il recupero delle quantità di analita aggiunto. Indici del livello di prestazioni sono stati introdotti al fine di stimolare il miglioramento della qualità dei risultati.

Parole chiave: elementi in traccia, valutazione esterna di qualità, fluidi biologici, indici del livello di prestazioni, Gran Bretagna.

Introduction

The Guildford external quality assessment scheme (EQAS) for trace elements in biological fluids was established in 1979 with distribution of specimens on a monthly schedule to United Kingdom hospital laboratories known to measure copper and zinc in serum. This was an informal arrangement initiated and funded by the Supra-regional Assay Service (SAS) Trace Element Laboratory, a facility run jointly by the Robens Institute of the University of Surrey and the Department of Clinical Biochemistry, Royal Surrey County & St. Luke's Hospitals, Guildford. During the next five years the scheme developed with inclusion of other participants (UK and overseas) and the introduction of additional analytes and specimen types. Eventually these developments were such that the costs could no longer be borne by the Trace Element Laboratory and in 1984 a participation charge was introduced to allow the employment of a full time member of staff. In 1986, a two year arrangement was established with the Commission of the European Community to fund participation in the serum aluminium programme for European laboratories involved with the monitoring of patients with chronic renal failure [1]. An outcome of this work was the realisation that analytical standards of performance for this measurement were very poor. In collaboration with the UK Department of Health it was proposed that the scheme should be linked to UK NEQAS in order to provide a mechanism for referral of UK National Health Service laboratories identified as poor performers, to the National Quality Assurance Advisory Panel for Chemical Pathology. This link was formally established in 1988.

To follow the significance of these steps it is helpful to understand the background and current organization of EQAS in the UK. The United Kingdom national external quality assessment scheme (UK NEQAS) was set up and funded by the Department of Health to organise external quality assessment for general clinical chemistry laboratories working within the National Health Service. Schemes for other disciplines and for some specialist analyses were later developed as part of the UK NEQAS organization. The trace elements EQAS became linked to UK NEQAS in 1988 as described above, but did not receive any government funding. With recent changes to the UK National Health Service there is no longer any central funding and all schemes have to secure their own

income by subscription from participants. The Chemical Pathology Advisory Panel, was formed a number of years ago by a Department of Health Working Party to give assistance to participants identified by UK NEQAS as having poor performance. Its members are appointed by professional associations involved in laboratory medicine and its activities are carried out in such a way as to protect the anonymity of participants. In addition to its supportive role to participants, the Advisory Panel has also been given the function of providing a formal "recognition" to EQAS. To be "recognised" by the Panel a scheme has to demonstrate that it meets certain technical, organisational and managerial criteria. The Guildford EQAS for trace elements in biological fluids was recognised by the Advisory Panel in 1993. Following changes to the UK National Health Service this function is about to be taken over by the organization responsible for the accreditation of clinical laboratories in the UK, Clinical Pathology Accreditation Ltd. EQA schemes will apply to CPA Ltd. for inspection and accreditation.

Organization

Objectives

When it was first established the primary functions of the scheme were to be educational and to stimulate improvements in analytical performance [2, 3]. These are still the most important roles but an increasing emphasis is directed towards the setting of performance standards. A possible development of this trend could be the certification of laboratories, where these standards are attained.

Steering committee

The activities of the scheme are supervised by a steering committee comprising individuals with acknowledged expertise in the area of trace element analysis, users of the scheme, and a representative from the Advisory Panel. The committee identifies objectives for the EQAS and monitors the action of the organisers in working towards these goals. Specifically, the committee sets targets indicative of poor and acceptable performance, determines assays which are thought to require particular attention because there are a large number of poor performers, and develops initiatives to stimulate improvements among participants or suggest the withdrawal of an assay from a laboratory's repertoire.

Participants

There are a total of 172 participants (in 1995) with 70 from UK laboratories and 102 situated in 25 other countries from throughout the world. Most, 108, are hospital laboratories with 23 private clinical laboratories, 19 occupational health departments, 14 university and veterinary, 6 industrial and 2 government laboratories. The scheme is open to any laboratory with an interest in measurement of trace elements. Most have a regular workload and are able to report results according to the schedule of the scheme. However, laboratories with an irregular routine will be accommodated, while for projects of limited duration a set of pre-assayed specimens with defined concentrations can be provided.

Analytes and specimens

The scheme has on-going programmes for the following analytes and matrices: aluminium, copper, gold, selenium and zinc in serum; lead and cadmium in whole blood; mercury and cadmium in urine; aluminium in dialysis fluids and water.

An area of current development is to increase the range of analytes included for urine and whole blood. A survey among participants indicated a need for EQA for additional elements e.g. arsenic, chromium, nickel, copper, zinc, iron. Specimens have been distributed in recent months with good response and a preliminary indication of the level of performances. It is anticipated that these analytes will soon become included as part of the main scheme.

Strategy for monitoring performance

The scheme is organised with a series of six-monthly cycles. At the start of a cycle a single batch of an appropriate matrix (e.g. horse serum) is divided into nine pools. The concentrations of analytes are increased in eight of the pools by addition of calculated amounts of standard solutions (BDH spectrosol solutions). The pools are then dispensed into tubes for subsequent distribution to participants. Three specimens are analysed every month and each pool is sent for analysis on two different occasions. Thus, the six-monthly cycle provides 18 specimens with duplicate measurements on the 9 pools. At the end of a cycle results from a participant for each matrix-analyte combination are evaluated for: proximity to the assigned value (18 data points), difference between results for duplicate measurements (9 data points), recovery of added analyte (8 data points).

Procedures

Sample preparation

All glassware is cleansed by soaking in 10% v/v HCl and rinsing with deionised, reverse osmosis-purified water. Tubes used for storage and distribution of specimens are of plastic, batches are screened before use [4] and found not to cause contamination to the contents.

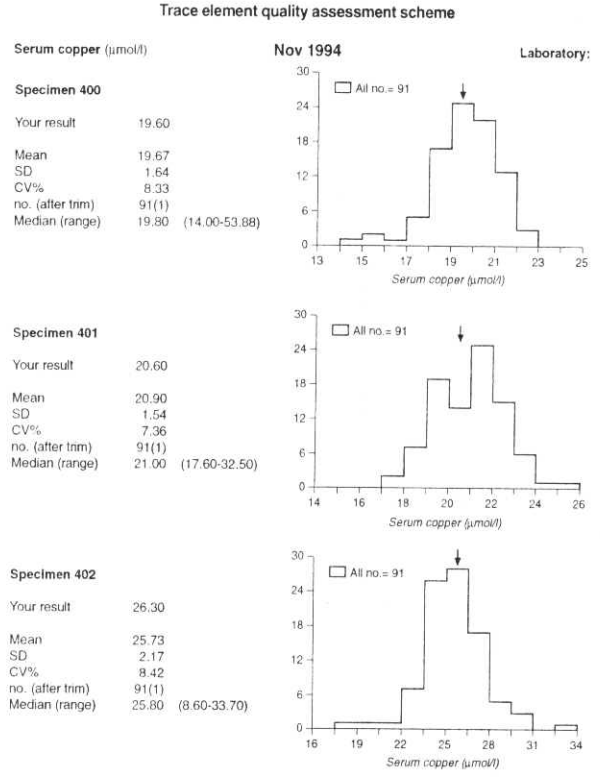

Fig. 1. - A page from the unique monthly report prepared for each participant.

Serum. - Test samples of sterile bovine or equine serum are provided by the supplier (Sera-Lab Ltd., Crawley Down, Sussex, UK) and screened to determine batches with low endogenous concentrations of trace elements. Two distinct preparations are manufactured. For aluminium and selenium EQAS, 4.5 l serum from a suitable batch is obtained and taken into nine 500 ml volumetric flasks for the addition of standard solutions to increase the analyte concentrations as required. For zinc EQAS, a specimen with low endogenous concentration is not usually available. Therefore, the trace elements in 4.5 l serum are removed by addition of Chelex 100 ion-exchange resin, 50 g/l, and the serum stirred continuously for 24 h. The suspension is centrifuged and the clear serum decanted into 500 ml volumetric flasks for addition of zinc, copper and gold. Manufactured samples are mixed and dispensed into labelled tubes.

Blood. - Human blood samples, 500 ml, are collected from three volunteers shown to be negative for Australia antigen and HTLV III antibodies, sonicated to disrupt the red cells and transferred to volumetric flasks for supplementation with lead and cadmium. The blood samples are mixed and dispensed into labelled tubes.

Urine. - Human urine, 2.5 l, collected during day time from a volunteer shown to be negative for Australia antigen and HTLV III antibodies is placed into volumetric flasks and the concentrations of mercury and cadmium augmented as for elements in serum or blood. The samples are mixed and dispensed into labelled tubes.

Dialysis fluids and water. - Dialysis fluid concentrate (Renalyte, Macarthays Medical Ltd., Romford, UK) 28.5 ml, is pipetted into a 1 l volumetric flask containing aluminium-free water. Nitric acid, 10 ml, and a solution of aluminium to increase the final concentration by a predetermined amount, are added. The solution is made to volume with water and thoroughly mixed. Water specimens are similarly prepared except for omission of the dialysis concentrate [5].

Serum, blood and urine specimens 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 [3].

Data reduction and reports

Each month the results reported by participants are entered into a computer programme for calculation of the mean and standard deviation for each of the matrix/analyte/specimen combinations. Results that fall outside of a range, mean ± 3 SD, are excluded from further computations. The trimmed data are used to determine means, medians, standard deviations and coefficients of variation. A unique report is prepared for each participant which shows these calculations and also gives their own results and histogram displays of the distributions of results (Fig. 1). At the end of a six-monthly cycle a review of performance (see below) is prepared for each matrix/analyte combination, for each laboratory.

Assessment of performance

Prior to 1990 the six-monthly review was the only assessment of performance given from the trace elements EQAS. In this report calculations of proximity to the target concentration (consensus median), recovery of added analyte and difference between results of specimens analysed on two separate occasions (x-X, %R, x1-x2) are considered. These data points are evaluated by reference to "target zones" as shown in Fig. 2. The zonal targets were prepared empirically to take account of the performance needed for clinical purposes and what can be achieved with current analytical techniques. The example shown in Fig. 2 is for aluminium in serum. Outer limits are selected at low and high levels (± 0.4 µmol/l at 1.0 µmol/l and ± 0.8 µmol/l at 4.0 µmol/l in this example for aluminium) and these are joined to give limits that cover all concentrations. An inner target zone is defined at half the outer limits. A key feature of this chart is an increased range, when expressed as a percentage

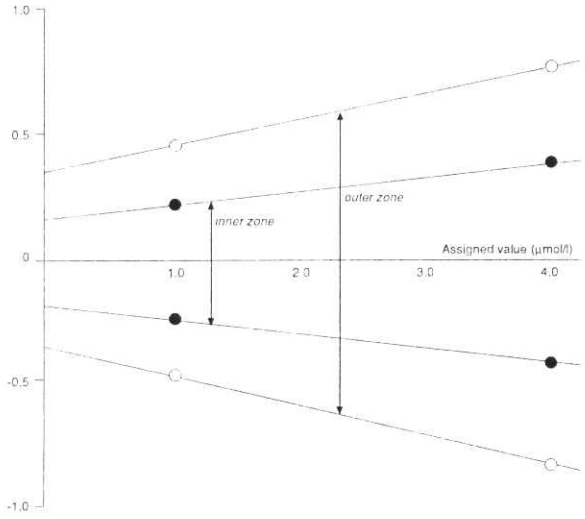

Fig. 2. - A zonal chart used for the assessment of performance. The example here is for aluminium in serum.

Table 1. - Limits used for each matrix-analyte combination to prepare zonal charts for assessments of accuracy and precision

Assay	Units	Inner limits	Outer limits
Serum aluminium	µmol/l	0.2 at 1.0 0.4 at 4.0	0.4 at 1.0 0.8 at 4.0
Serum copper	µmol/l	0.5 at 4.0 0.75 at 20.0	1.0 at 4.0 1.5 at 20.0
Serum gold	µmol/l	0.25 at 4.0 0.6 at 16.0	0.5 at 4.0 1.2 at 16.0
Serum selenium	µmol/l	0.06 at 0.75 0.1 at 2.0	0.12 at 0.75 0.2 at 2.0
Serum zinc	µmol/l	0.5 at 4.0 0.75 at 20.0	1.0 at 4.0 1.5 at 20.0
Blood cadmium	nmol/l	4 at 50 7.5 at 200	8 at 50 15 at 200
Blood lead	µmol/l	0.07 at 0.48 0.12 at 2.9	0.14 at 0.48 0.24 at 2.9
Urine cadmium	nmol/l	5 at 50 12 at 300	10 at 50 24 at 300
Urine mercury	nmol/l	5 at 50 15 at 500	10 at 50 30 at 500
Dialysis fluids/water aluminium	µg/l	5 at 27 11 at 108	11 at 27 22 at 108

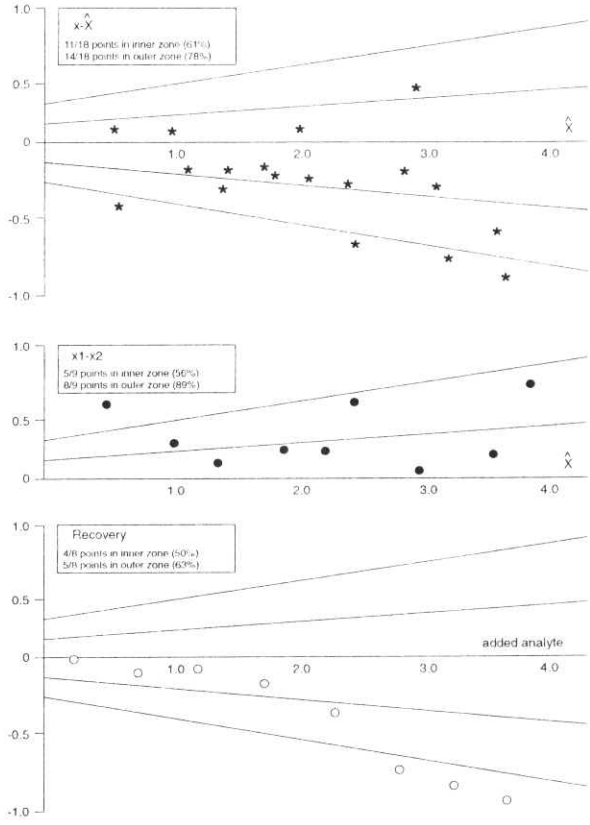

Fig. 3. - Graphical display of one participant's performance throughout a six-month cycle, used to calculate the performance score (top: proximity to the assigned value; middle: difference between results for duplicated samples; bottom: recovery of added analyte).

of the assigned value, at concentrations close to the analytical detection limit. The limits used to prepare zonal charts for the different matrix-analyte combinations in the scheme are given in Table 1 [3].

A plot of data points for one participant's six-monthly serum aluminium review is shown in Fig. 3 and reveals that this laboratory had good precision but there was a systematic bias. These data and the plots are also used to determine a "performance score". The percentage of points falling within the inner and outer zones of the x-X chart are calculated (61 and 78% in Fig. 3 - top). Similar calculations are made for the %R and x1-x2 charts, all six percentages are added together and the total is divided by 6 to give the performance score. The maximum sum is 600 and a laboratory will, therefore, have a performance score of up to 100 (the performance score for the laboratory shown in Fig. 3 is 66). Scores permit the comparison of one participant against others and show trends in performance with time, therefore they are valuable for promoting improvements [6].

As these performance assessments and scores are made every six months they are inevitably somewhat out of date to be of immediate benefit to participants. A revised system was introduced in 1990, with the introduction of *monthly* and *cumulative* scores based only on the proximity of a result to the assigned value. For each specimen assayed the proximity of a participant's result to the assigned value is plotted onto a zonal chart, as shown in Fig. 4.

A point between the inner limits gains a score of 2, a point between the inner and outer limits scores 1 while anything outside the limits has a 0 score. Each month there are 3 samples so that a good performance is indicated by a *monthly* score of 6. The *cumulative* score is the sum of the most recent 6 monthly scores and will have a maximum of 36. An example of the report for monthly and cumulative scores is shown in Fig. 5.

Standards of performance

It was suggested by the scheme organisers that for UK laboratories there should also be standards of performance associated with these scores. A proposal that, for aluminium in serum, a cumulative score of 30 or more is indicative of acceptable performance and that a score of below 25 is unacceptable, was agreed by the Steering Committee. Equivalent standards for zinc and copper in serum were set at cumulative scores of 20 and 10. It was also agreed that if a participant had an unacceptable cumulative score in a sequence of three out of four months, this would be reported to the UK Advisory Panel for Quality Assurance.

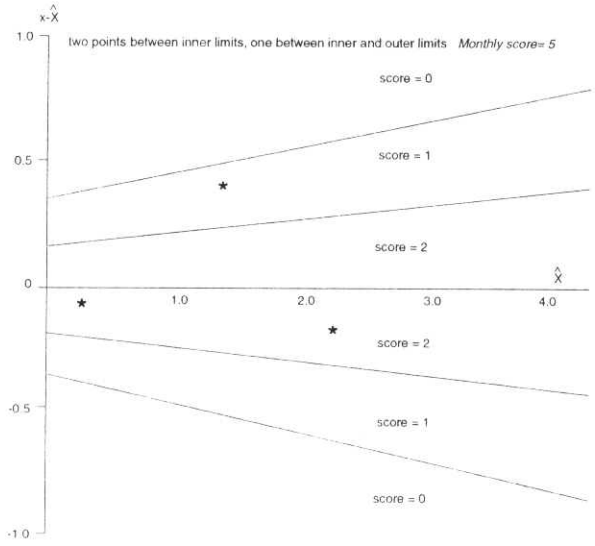

Fig. 4. - Use of the zonal chart for the determination of a monthly score. The example here is for aluminium in serum. For each of the three results obtained by the participant, the proximity to the assigned value is plotted at the appropriate concentration.

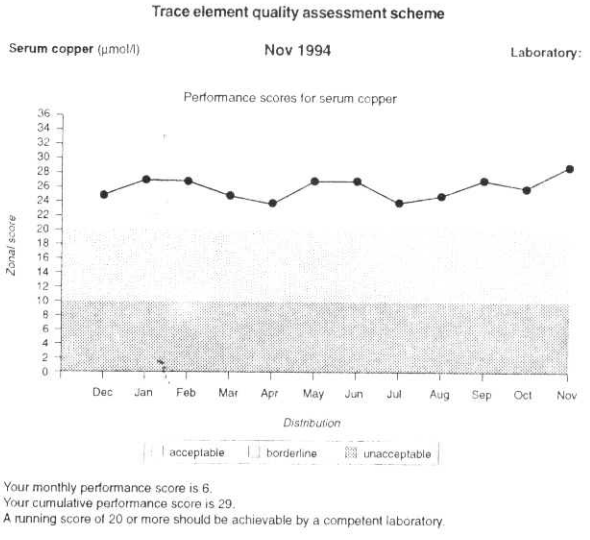

Fig. 5. - A page from the unique monthly report prepared for each participant to show the monthly and cumulative scores and the targets for acceptable and unacceptable performance.

Results of the scheme and other considerations

The organization of the Guildford trace elements EQAS is consistent with the recommendations of the American Official Association of Chemistry (AOAC), the International Organization for Standardisation (ISO) and the International Union of Pure and Applied Chemistry (IUPAC) [7]. Two aspects relevant for further discussion are the determination of assigned values and the calculation of the monthly score. Assigned values are prepared from participant consensus data rather than from the results given by reference laboratories. Experience from our own work and from other schemes has shown that there is good agreement between consensus values and the amounts added during preparation of the specimens, or with assigned values determined by reference laboratories [3]. The international recommendations [7] propose the determination of a "z-score" for performance assessments. Typically the z-score is derived from the proximity of the result (x) to the assigned value (X) divided by the standard deviation [z = (x-X)/SD] but variations to this approach are acknowledged as appropriate to certain assays. The zonal limits shown in Fig. 2 and Table 1 were developed empirically but it has been shown that these are close to the between-laboratory precision profiles that can be drawn for each assay using the scheme data (unpublished results). Thus, our zonal scores are equivalent to the z-scores and we are now experimenting with ways to make this more explicit to users [8].

A number of initiatives to stimulate improved performance among participants have been undertaken both nationally and internationally. These have included

provision of materials for internal quality control, comparisons of methodological approaches, meetings for participants, scientific conferences. These initiatives have met with considerable success [9].

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Appendix. - Summary of the scheme

Country	United Kingdom.
Name of scheme	Guildford trace elements external quality assessment scheme (TEQAS).
Status of scheme	International, national (UK NEQAS) voluntary. Run by Robens Institute, University of Surrey and Department of Clinical Biochemistry, Immunology and Nutrition, Royal Surrey County Hospital, Guildford,UK. *Aims:* educational and to stimulate improvements in analytical performance. *Participants:* 172 (70 within UK and 102 in 25 other countries). Hospital laboratories: 108; private clinical laboratories: 23; occupational health departments: 19; university and veterinary laboratories: 14; industrial organizations: 6; government facilities: 2.
Scheme description	*Control materials:* prepared in the laboratory from bovine/equine serum, human whole blood and urine. Addition of known amounts of analytes to liquid pools. Liquid, multi-element samples, in plastic vials. Target value chosen as the consensus median of laboratories' results after exclusion of outliers (i.e. results falling outside the range mean ± 3 SD). Internal quality control samples or calibrators are not distributed as a formal component of the scheme. Some RMs are manufactured for participants to use as routine IQC samples. *Organization of EQA exercises:* monthly distribution with 3 specimens per month, within a 6-month cycle. Samples sent at beginning of month, results should be returned by last day of month by fax, post or telephone.. *Elaboration of results:* computer program to calculate means, medians, standard deviations, coefficients of variation and histogram displays of the distributions of results. *Criteria for evaluation of laboratory performance:* a) comparison of laboratory results at the end of each six-monthly cycle with target zones for proximities to assigned values, difference between results for duplicate measurements and recovery of added analyte; b) monthly and cumulative scores derived from proximity of results to the assigned values (equivalent to "z-scores"). Standards for acceptable and unacceptable performance have been developed. *Measures taken against poor performers:* a) all laboratories are informed with the monthly report as to whether current performance is acceptable or unacceptable; b) For UK NEQAS participants only, if poor performance continues, a personal letter is sent to the head of department to encourage corrective action and to offer advice and support. Where problems persist the situation is reported (anonymously) to the National Quality Assurance Advisory Panel for Chemical Pathology. *Advice, assistance and training:* offered to poor performers (see above) and previously assayed samples are made available to help investigate problems. Personnel visit other laboratories in the UK and abroad for ad hoc teaching, training and problem-solving purposes but there is no formal training at this time. *Financial support:* from participant subscriptions.
Organization	Robens Institute, University of Surrey, Guildford GU2 5XH, United Kingdom and Department of Clinical Biochemistry, Immunology and Nutrition, Royal Surrey County Hospital, Guildford GU2 5XX, United Kingdom Dr Andrew Taylor Tel + 44 1483 502742 Dr Raymond J Briggs Tel + 44 1483 259217 Fax + 44 1483 503517 e-mail A.Taylor@surrey.ac.uk
Analytes and matrices covered	*Established* Serum: aluminium, copper, gold, selenium, zinc. Whole blood: lead, cadmium. Urine: mercury, cadmium. Dialysis fluid, water: aluminium. *Under development* Whole blood, urine: arsenic, chromium, copper, cobalt, iron, magnesium, manganese, nickel, zinc.