

X Series ICP-MS Clinical Applications Note 5: Trace element quantification in blood and serum in a single analytical run.

Key Words

- Blood
- Serum
- CCT
- Copper
- Zinc
- Selenium
- Cadmium
- Lead

Introduction

Elemental analysis in clinical samples has received increasing attention in recent years as knowledge of the pathological affects of certain element deficiencies and excesses has grown. Consequently, the demands on analytical instrumentation to address the challenges involved in trace element measurement in clinical samples have also increased. Historically, graphite furnace AAS has been the technique of choice in this field, owing to its ability to accurately measure many analytes at trace levels in a range of sample matrices, and in many cases, graphite furnace AAS remains the technique of choice. However, relatively long analysis times, limited multi-element capability and complex, analyte dependent sample preparation requirements (such as the use of chemical modifiers to stabilise target elements during the ashing process or to promote vaporisation in the measuring stage) have led analysts to investigate the potential of ICP-MS for some of their applications. With the advent of collision and reaction cell technology for ICP-MS, the interference problems that previously limited the capability of this technique for measurement of Se in particular (Ar^{2+} interferes with ^{76}Se , ^{78}Se and ^{80}Se) in clinical samples have been largely overcome. This innovation, together with improvements in sample introduction for complex matrix materials such as blood and serum, as well as the fast, multi-element capabilities of the technique, has led to ICP-MS rapidly gaining popularity in the clinical field.

Serum has long been known to be a useful material in which to measure elements such as Cu, Zn and Se for diagnosis and monitoring of certain diseases and for nutritional studies. It is a complex matrix substance that consists of inorganic salts and a high concentration of protein (approximately 70 g/l), together with a variety of trace substances. Copper, zinc and selenium are all essential elements in the body, but equally, if they are present in excess, toxic effects result. The typical levels for these three elements in the serum of healthy adult individuals are: Cu - $17 \mu\text{mol L}^{-1}$ ($1.1 \mu\text{g g}^{-1}$), Zn - $14 \mu\text{mol L}^{-1}$ ($0.9 \mu\text{g g}^{-1}$) and Se - $2 \mu\text{mol L}^{-1}$ ($0.16 \mu\text{g g}^{-1}$). Elevated copper levels in serum are one indication of Wilson's disease, an autosomal recessive disorder affecting around 1 in 100,000 people, first identified by Kinnear-Wilson in 1912. With this condition, the rate of copper incorporation into caeruloplasmin and its biliary excretion

are both reduced, leading to accumulation of copper in the liver, parts of the brain and the eye. Symptoms of the disease include liver cirrhosis, neurological damage (leading to muscle spasms and tremor), and Kayser-Fleischer rings (rings of copper deposits in the cornea). Without treatment, the condition is ultimately fatal. Copper deficiency on the other hand can lead to inefficient metabolism of iron and protein and stunted growth.

Ingestion of excess quantities of Zn causes vomiting, muscle co-ordination problems and dizziness, whereas zinc deficiency has been identified as the cause of anaemia and growth retardation in populations whose diet is deficient in this element. Zinc is known to be involved in the functioning of the immune system and has been associated with the operation of more than 200 enzymes, some of which are related to DNA and RNA synthesis.

Acute Se poisoning has been reported to cause irritation of the eyes, nose, mouth and lungs, followed by nausea, abdominal pain and in very severe cases cardio-respiratory arrest. Another well documented symptom is a garlic odour on the breath of the victim. Chronic selenium depletion has been implicated as an increased risk factor for cancer, cardiovascular disease and stunted bone growth, and has been identified as the cause of Kashin-Beck disease, an osteoarthropathy condition, particularly prevalent in Shaanxi province, China, where Se has been found to be highly deficient in the diet.

Compared to serum, whole blood has a more complex composition. It is composed mainly of water (90% by volume), red blood cells (erythrocytes), platelets (thrombocytes), several different types of white blood cells (leukocytes), and proteins, such as albumin and fibrinogen. The aqueous part is sub-divided into approximately 55% plasma and 45% red blood cells. Within this solution, a number of major and trace elements are also present, ranging in concentration from a few nmol L^{-1} to several mmol L^{-1} . Many of these elements, such as Na and K, are present as essential nutrients to maintain cell activity, but others, such as Pb and Cd, are non-essential, toxic elements that have entered the body via contaminated air, water or food. After ingestion, heavy metals often become predominantly associated with haemoglobin and for this reason whole blood is particularly useful for monitoring occupational exposure to contaminants such as Pb and Cd. For Pb, current US legislation established by the Occupational Safety and

Health Administration (OSHA), states that the upper allowable limit of this element in blood is 500 ng mL⁻¹. If an exposed individual is found to have Pb levels in excess of this, they are moved from the exposure environment until their blood Pb level falls below 400 ng mL⁻¹. In Europe, the maximum allowable Pb level in blood is 600 ng mL⁻¹. In cases where significant exposure to Pb is identified, the patient may be treated with a complexing agent such as calcium disodium EDTA or penicillamine, which expedites excretion of Pb from the body.

Cadmium can enter the body as a result of industrial exposure from activities such as steel production, pigment and battery manufacturing or from dietary sources such as rice, wheat, oysters and other seafoods. Also, cigarettes contain significant levels of cadmium and this is reflected in the elevated Cd levels observed in smokers (> 80 nmol L⁻¹) compared to non-smokers (< 20 nmol L⁻¹), (for non-occupationally exposed individuals). As with Pb, upon entering the body, Cd becomes mainly bound to erythrocytes in the blood and accumulates in liver and kidney tissue. In terms of its toxicity, acute air exposure to Cd can cause respiratory distress, nausea and vomiting. Chronic exposure to Cd leads to kidney damage, brittle bones (osteomalacia) and considerable pain, as evidenced by the well-known case of industrial Cd poisoning along the Jintzu River basin area in the Toyama Prefecture, Japan. This incident, caused by use of irrigation water polluted with cadmium from a zinc processing plant, was linked to multiple cases of Itai-Itai disease (literally 'ouch-ouch', in reference to the pain caused by the illness). However, malnutrition and vitamin deficiency were also implicated as factors contributing to the disease.

For this applications note, samples prepared for the UK NEQAS EQA Cu, Zn and Se in serum and Pb and Cd in blood schemes were used. The UK NEQAS (United Kingdom National External Quality Assessment Service) is a non-profit organisation whose aim is to 'advance education and promote the presentation of good health by providing external quality assessment services for clinical laboratories'. This aim is principally achieved by the organisation, administration and result reporting of round robin schemes set up to generate consensus values for a variety of analytes in clinical samples.

This application note describes the performance of the X Series ICP-MS from Thermo Electron Corporation for measurement of Cu, Zn and Se in serum and Cd and Pb in whole blood, in a single analytical run.

Sample preparation

Ten serum and twelve whole blood samples from the UK NEQAS were supplied for analysis. All samples were diluted 1:50 with the custom clinical diluent described previously¹ in application note AN_0601. Ga, Ge, Rh, Re and Tl (at 10 ng mL⁻¹) were used as internal standards. In the analysis, the serum samples were measured first, followed by the blood samples, with all samples being measured in a single run. In addition to the samples, blood and serum reference materials (Seronorm serum and Seronorm blood levels 1 and 2) were also analysed after reconstitution from the freeze-dried

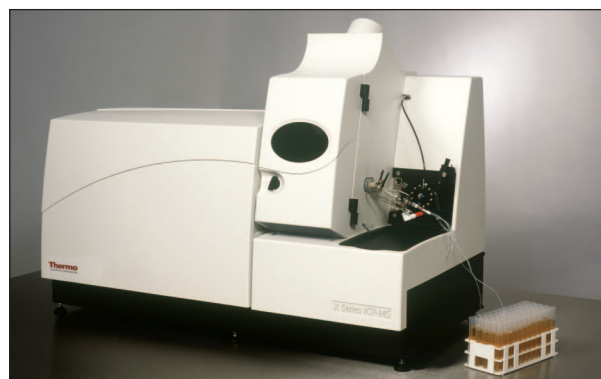
material and subsequent 1:50 dilution with the same diluent used to dilute the samples.

Calibration solution preparation

Two point external calibration solutions were prepared at appropriate concentrations by serial dilution of parent 1000 µg mL⁻¹ stock solutions of Cu, Zn, Se, Cd and Pb, using the same diluent used to dilute the samples.

Instrument configuration

The instrument was operated in standard and collision cell modes (using 8% (v/v) H₂ in He as the collision gas), with in-sample switching between the two modes. Standard (non-CCT) mode is the preferred method for acquiring data for ⁶⁴Zn, ⁶⁵Cu, ⁶⁶Zn, ⁸²Se, ¹¹¹Cd and ²⁰⁸Pb and CCT mode is the better approach for measuring the ⁶³Cu and ⁸⁰Se isotopes as it eliminates the ⁴⁰Ar²³Na⁺ and ⁴⁰Ar₂⁺ interferences, respectively. The instrument was operated with a Peltier cooled impact bead spray chamber, single piece quartz torch (1.5 mm i.d. injector) and the enhanced sensitivity PlasmaScreen Plus option, together with Xi interface cones. A Burgener Miramist high solids parallel path nebuliser (part number 4600356) was used, as this device does not block during aspiration of clinical samples and can be run at lower sample uptake rates (down to 0.4 ml min⁻¹) than conventional glass concentric nebulisers.



Instrument parameters

The instrument was operated using the following parameters:

RF power:	1350 W
Nebuliser gas flow:	0.87 L min ⁻¹
Auxiliary gas flow:	0.60 L min ⁻¹
Cool gas flow:	13.0 L min ⁻¹
Isotopes measured:	⁶³ Cu, ⁶⁴ Zn, ⁶⁵ Cu, ⁶⁶ Zn, ⁷¹ Ga*, ⁷⁴ Ge*, ⁸⁰ Se, ⁸² Se, ¹⁰³ Rh*, ¹¹¹ Cd, ¹⁸⁷ Re*, ²⁰⁵ Tl* and ²⁰⁸ Pb

(* = internal standard for standard mode operation and ** = internal standard for CCT mode operation)

Dwell time per isotope: 10 ms

Sample uptake and wash time: 60 s

Sample acquisition time:

Main run (peak jumping) = 24 s per repeat (3 repeats per sample)

In sample switching time between standard and CCT mode = 30 s per sample

Total acquisition time: 2 min 54 s

Results and discussion

For the sample analysis, in-sample analysis mode switching between standard and CCT mode was used. With the X Series ICP-MS, stable and reproducible automatic switching between different operation modes can be easily achieved, with a settle time of only 30 seconds to allow all the parameters to adjust to their required values and for the analyte signals to stabilise. Figure 1 below illustrates how reproducibly and rapidly the X Series can automatically switch from standard operation to CCT mode and back to standard again, for each sample in a run. The figure shows signal count rates for 3 repeat measurements of a standard measured at the start of the run (run 1: red, yellow and blue columns) and the same standard measured again at the end of the run (run 2: green, pink and black columns). The relative standard deviations (% RSD's), shown above each isotope data set correspond to the two blocks of 3 repeats (run 1 and run 2).

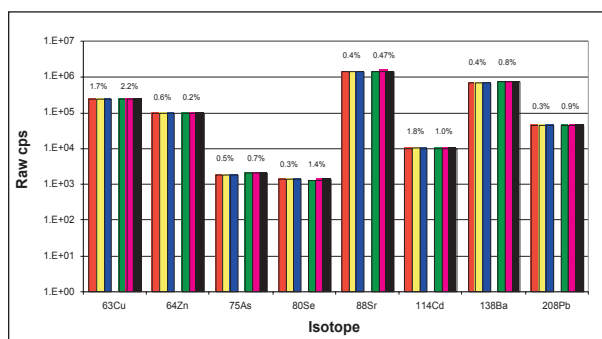


Figure 1. Reproducibility of in-sample switching.

Serum analysis results

The Cu results obtained for the ten serum samples and the Seronorm reference serum, compared to the consensus / reference values, (using the ^{63}Cu data) are presented in Table 1.

SAMPLE IDENTITY	MEASURED ^{63}Cu VALUE ($\mu\text{mol L}^{-1}$)	CONSENSUS / REFERENCE Cu VALUE ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	35.9 ± 0.2	38.1 ± 4.3	94
677	7.93 ± 0.03	8.9 ± 1.4	90
678	14.7 ± 0.1	16.2 ± 1.4	91
679	29.6 ± 0.2	32.1 ± 2.6	92
680	24.3 ± 0.4	26.9 ± 2.3	90
681	19.3 ± 0.2	20.8 ± 2.0	93
682	13.5 ± 0.1	14.7 ± 1.3	92
683	10.8 ± 0.1	11.8 ± 1.4	92
684	16.6 ± 0.2	17.9 ± 1.5	92
685	8.00 ± 0.06	8.64 ± 1.12	93
Seronorm serum	17.4 ± 0.08	18.6 ± 0.6	94

Table 1. Consensus Cu in serum values versus X Series ICP-MS results (± 1 standard deviation).

To illustrate the accuracy and precision achieved with the X Series ICP-MS using the in-sample switching approach, the measured results obtained for both Cu isotopes (^{63}Cu - CCT mode, ^{65}Cu - standard mode) are compared below (Table 2).

SAMPLE IDENTITY	^{63}Cu RESULT ($\mu\text{mol L}^{-1}$) *	^{65}Cu RESULT ($\mu\text{MOL L}^{-1}$) **
676	35.9 ± 0.2	36.1 ± 0.1
677	7.93 ± 0.03	7.83 ± 0.09
678	14.7 ± 0.1	14.6 ± 0.1
679	29.6 ± 0.2	29.6 ± 0.4
680	24.3 ± 0.4	24.6 ± 0.5
681	19.3 ± 0.2	19.0 ± 0.2
682	13.5 ± 0.1	13.6 ± 0.1
683	10.8 ± 0.1	10.7 ± 0.1
684	16.6 ± 0.2	16.5 ± 0.1
685	8.00 ± 0.06	8.17 ± 0.15
Seronorm serum	17.4 ± 0.08	17.7 ± 0.04

* collision cell (CCT) operation

** standard mode operation

Table 2. Comparison of ^{63}Cu and ^{65}Cu data for the serum analysis (± 1 standard deviation).

Excellent agreement between the results for each isotope was achieved, highlighting the stable performance achieved during in-sample switching between standard and collision cell operation, although use of the ^{63}Cu isotope is preferred as this is the more abundant and therefore more sensitive Cu isotope, even when the slight sensitivity reduction observed in CCT mode is taken into account.

The Zn results obtained for the ten serum samples, compared to the consensus values, are presented in Table 3. In this table, X Series data for ^{64}Zn are presented, as this is the most abundant isotope available and in this work, no interference from ^{64}Ni or sulphur based interferences were encountered. The results for the lower abundance ^{66}Zn isotope were consistent with the ^{64}Zn data.

SAMPLE IDENTITY	MEASURED ^{64}Zn VALUE ($\mu\text{mol L}^{-1}$)	CONSENSUS / REFERENCE Zn VALUE ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	3.24 ± 0.03	3.61 ± 1.07	90
677	2.18 ± 0.06	2.48 ± 1.12	88
678	15.97 ± 0.05	16.43 ± 1.99	97
679	7.56 ± 0.03	8.14 ± 1.36	93
680	10.5 ± 0.3	10.8 ± 1.4	97
681	8.9 ± 0.1	9.3 ± 1.8	95
682	23.2 ± 0.1	23.2 ± 2.6	100
683	31.2 ± 0.2	31.3 ± 3.4	99
684	13.2 ± 0.2	13.7 ± 1.3	97
685	2.01 ± 0.03	2.40 ± 0.90	84
Seronorm serum	18.7 ± 0.3	20.3 ± 0.8	92

Table 3. Consensus Zn in serum values versus X Series ICP-MS results (± 1 standard deviation).

Good agreement between the measured and consensus results for Zn in the serum samples was obtained.

The selenium results obtained for the ten serum samples supplied, compared to the consensus values are presented in Table 4(a).

SAMPLE IDENTITY	MEASURED VALUE ($\mu\text{mol L}^{-1}$)	CONSENSUS VALUE ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	0.43 ± 0.01	0.29 ± 0.09	148
677	1.66 ± 0.05	1.48 ± 0.12	112
678	3.70 ± 0.07	3.30 ± 0.34	112
679	1.45 ± 0.03	1.21 ± 0.12	120
680	0.73 ± 0.02	0.58 ± 0.08	126
681	2.50 ± 0.03	2.34 ± 0.24	107
682	1.09 ± 0.02	0.87 ± 0.12	125
683	2.01 ± 0.02	1.76 ± 0.15	114
684	3.10 ± 0.04	2.66 ± 0.35	117
685	0.42 ± 0.01	0.28 ± 0.07	151
Seronorm serum	1.04 ± 0.02	1.05 ± 0.04	99

Table 4(a). Selenium in serum results (± 1 standard deviation), 1:50 dilution corrected.

Table 4(a) shows that poor agreement was achieved between the measured and consensus values for Se in serum, with the exception of the Seronorm reference sample. In each case, the X Series results were consistently higher than the corresponding consensus results that were obtained using graphite furnace AAS. Further investigations were performed to identify which set of data were correct. Initially, a freshly prepared set of samples were run on a PlasmaQuad 3 ICP-MS (PQ3) at the University of Liverpool, using independently prepared calibration solutions. This instrument was not equipped with a collision cell, so it was necessary to make measurements using the less sensitive (and least interfered) ^{82}Se isotope, with interference correction for ^{82}Kr applied in the software. The results achieved compared to the X Series ICP-MS are shown in Table 4(b).

SAMPLE IDENTITY	X SERIES RESULT ($\mu\text{mol L}^{-1}$)	PQ3 RESULT ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	0.43 ± 0.01	0.43	100
677	1.66 ± 0.05	1.63	102
678	3.70 ± 0.07	3.51	105
679	1.45 ± 0.03	1.38	105
680	0.73 ± 0.02	0.73	100
681	2.50 ± 0.03	2.52	99
682	1.09 ± 0.02	1.08	101
683	2.01 ± 0.02	1.92	105
684	3.10 ± 0.04	2.83	110
685	0.42 ± 0.01	0.44	96

Table 4(b). Selenium in serum - PQ3 versus X Series ICP-MS results (± 1 standard deviation), 1:50 dilution corrected.

The data presented in Table 4(b) show that excellent agreement between the results of these two independent ICP-MS analyses was achieved, although the X Series CCT option offered lower detection capability and enhanced measurement precision and accuracy at lower

Se concentrations than the PQ3 because the more abundant ^{80}Se isotope could be used. These results showed consistency in the ICP-MS measurement approach but still did not confirm which method generates the more accurate data; ICP-MS or GFAAS. So, an additional experiment was performed using a GFAAS system at the University of Liverpool to produce a comparison data set. The consensus values reported by the serum analysis round robin program are often obtained using a procedure based on standard additions of an inorganic selenium standard to sub-samples of the serum material. There is therefore a question as to whether the added inorganic selenium behaves in the same way as any organic selenium that may be present in the serum, during the ashing step of the GFAAS analysis in particular. It was hypothesised that if some Se in the serum was present as organo-selenium species these species may not react with the matrix modifier during the ashing step and be lost before the atomisation step. This would produce consistently low furnace results compared to ICP-MS measurements, as was observed in this analysis. To try to compensate for any such effect, the procedure adopted in this experiment was to use two serum reference materials, certified for their Se content as the external calibration standards and to measure the samples relative to this calibration. The results achieved, compared to the X Series data are presented in Table 4(c).

SAMPLE IDENTITY	X SERIES RESULT ($\mu\text{mol L}^{-1}$)	GFAAS RESULT ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	0.43 ± 0.01	0.32	134
677	1.66 ± 0.05	1.63	102
678	3.70 ± 0.07	3.71	100
679	1.45 ± 0.03	1.47	98
680	0.73 ± 0.02	0.74	98
681	2.50 ± 0.03	2.66	94
682	1.09 ± 0.02	1.11	98
683	2.01 ± 0.02	2.25	89
684	3.10 ± 0.04	3.33	93
685	0.42 ± 0.01	0.4	106

Table 4(c). Selenium in serum X Series ICP-MS versus matrix matched calibration GFAAS results (± 1 standard deviation), 1:50 dilution corrected

Improved agreement was obtained between the GFAAS results achieved using the external calibration method described above and the X Series ICP-MS results. The exception was sample 676, for which the furnace result was significantly lower than the ICP-MS result. The agreement obtained in this experiment was considerably better compared to the corresponding comparison with the consensus results, suggesting that the ICP-MS approach may provide the more accurate results for Se in serum.

Blood analysis results

For the blood samples, consensus values were available for Pb and Cd, so these data were compared to the X Series ICP-MS results. The Pb results obtained for the twelve whole blood samples and two reference materials, compared to the consensus / reference values, are presented in Table 5 and the Cd results for these materials are presented in Table 6.

SAMPLE IDENTITY	MEASURED VALUE ($\mu\text{mol L}^{-1}$)	CONSENSUS / REFERENCE VALUE ($\mu\text{mol L}^{-1}$)	RELATIVE ACCURACY (%)
676	0.065 ± 0.002	0.09 ± 0.05	72
677	4.23 ± 0.09	3.68 ± 0.38	115
678	1.630 ± 0.005	1.57 ± 0.18	104
679	0.63 ± 0.02	0.61 ± 0.06	103
680	1.53 ± 0.01	1.47 ± 0.11	104
681	4.10 ± 0.02	3.78 ± 0.26	108
682	1.610 ± 0.008	1.59 ± 0.09	101
683	0.603 ± 0.009	0.61 ± 0.06	99
684	1.54 ± 0.04	1.47 ± 0.10	105
685	0.072 ± 0.001	0.08 ± 0.03	90
686	1.12 ± 0.01	1.05 ± 0.09	107
687	4.92 ± 0.07	4.53 ± 0.46	109
Seronorm whole blood 1	0.150 ± 0.002	0.16 ± 0.02	94
Seronorm whole blood 2	1.912 ± 0.004	1.93 ± 0.22	99

Table 5. Pb in whole blood consensus/reference values versus X Series ICP-MS results (± 1 standard deviation).

Good agreement between the measured and consensus results for Pb in the whole blood samples was obtained. Sample number 676 gave a lower result on the X Series compared to the consensus mean, but considering the large spread in results found for the consensus value, it appears that sample contamination may be a problem for GFAAS at this low Pb level. The results for the two blood reference materials were also consistent with the certificate values.

SAMPLE IDENTITY	MEASURED VALUE (NMOL L ⁻¹)	CONSENSUS / REFERENCE VALUE (NMOL L ⁻¹)	RELATIVE ACCURACY (%)
676	3.21 ± 0.70	5.26 ± 4.04	61
677	39.36 ± 1.31	35.97 ± 5.71	109
678	175.25 ± 7.93	159.17 ± 23.41	110
679	68.16 ± 3.01	65.14 ± 7.41	105
680	113.33 ± 3.61	103.98 ± 12.71	109
681	39.12 ± 2.78	37.33 ± 4.57	105
682	167.24 ± 5.34	149.36 ± 38.36	112
683	69.37 ± 2.30	66.69 ± 14.35	104
684	120.45 ± 4.18	103.17 ± 14.59	117
685	5.14 ± 1.08	4.57 ± 2.51	112
686	298.19 ± 17.45	250.5 ± 38.19	119
687	103.10 ± 4.07	89.37 ± 12.05	115
Seronorm whole blood 1	5.55 ± 0.15	6.22 ± 0.53	89
Seronorm whole blood 2	58.23 ± 2.24	55.15 ± 8.90	106

Table 6. Cd in whole blood consensus/reference values versus X Series ICP-MS results (± 1 standard deviation).

Reasonable agreement was obtained between the measured and consensus results for Cd in the whole blood samples, although for the majority of samples the X Series values were slightly higher than the consensus results. However, for all samples the X Series results lay within or overlapped the consensus standard deviation range (± 1 standard deviation), so this apparent bias may be a result of the spread in the consensus data. As with the Pb data, sample number 676 gave a lower result on the X Series compared to the consensus mean, but considering the large spread in results found for the Cd consensus value, it may be that accuracy is a problem for GFAAS measurements at this low Cd level. The results for the two blood reference materials were consistent with the certificate values within the ± 1 standard deviation range.

Conclusions

This application note has demonstrated that the X Series ICP-MS is an effective instrument for rapid, accurate and precise measurement of both minor and trace elements in both serum and blood samples, in a single analytical run. Both sample types can be prepared using a single, one-step dilution procedure based on a custom clinical diluent, designed to provide optimum analyte stability with minimum memory effects between samples. With this rapid and simple procedure, the X Series ICP-MS can routinely and cost – effectively analyse hundreds of samples per day at and considerably below the concentration levels currently required by clinical analysts. The flexible combination of the enhanced sensitivity PlasmaScreen Plus, collision cell technology (CCT) and Xi interface options, complemented by improved sample introduction systems means that ICP-MS is fast becoming the technique of choice for routinely quantifying minor and trace metals in clinical samples in both high throughput biomedical analysis laboratories and medical research facilities.

¹Application note AN_0601: Rapid and accurate measurement of As and Cr in urine.

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