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# A comparison of double-focusing sector field ICP-MS, ICP-OES and octopole collision cell ICP-MS for the high-accuracy determination of calcium in human serum

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**Abstract**

Human serum is routinely measured for total calcium content in clinical studies. A definitive high-accuracy and low-uncertainty method is required for reference measurements to underpin medical diagnoses. This study presents a novel octopole collision cell ICP-MS, high-accuracy, methodology and comparison of that technique with double-focusing sector field ICP-MS and an ICP-OES method. Double-matched isotope dilution mass spectrometry (IDMS) was employed for ICP-MS techniques and an exact matching bracketing technique using scandium as an internal standard was used for ICP-OES analysis. Medium resolution mode was utilised for double-focusing sector field ICP-MS analysis to resolve the dominant interferences on the $^{44}Ca/^{42}Ca$ isotope pair. Hydrogen reaction gas was employed to chemically resolve a number of polyatomic interferences predominantly through charge transfer reactions in the octopole collision cell. Comparison data presented for NIST CRM 909b human serum analysis from all three techniques demonstrates highest accuracy (99.6%) and lowest uncertainty (1.1%) for octopole collision cell ICP-MS. Data from ICP-OES using a non-IDMS technique produces comparably accurate data and low-uncertainties. The much higher total expanded uncertainties for double-focusing sector field ICP-MS compared with octopole collision cell data are explained by lower precision on the measurement of the $^{44}Ca/^{42}Ca$ isotope ratio. Data for octopole collision cell ICP-MS submitted for an international blind trial comparison (CCQM K-14) demonstrated excellent agreement with the mean of all participants with a low expanded uncertainty.


*Keywords:* High accuracy; Human serum; Octopole collision cell; IDMS

## 1. Introduction

Of the total calcium in the body 99% is found in bones and teeth with the remainder in soft tissue and body fluids where it regulates a number of normal body processes [1]. Beneficial Ca functions include: maintenance of healthy teeth and bones, vital component for blood clotting and as a wound-healing agent [1]. Calcium is essential for muscle contraction [2] and helps to control blood pressure and nerve transmission [1]. Calcium deficiency is responsible for an increased risk of hypertension [3,4], arteriosclerosis, alzheimers [5], colon cancer and premenstrual syndrome [4]. Loss of Ca from the bones can result in skeletal deformity [6], osteoporosis and other degenerative joint diseases [5]. Calcium serum levels are additionally measured as an indication of thyroid disease [4] such as primary and tertiary hyperthyroidism and as indicators of Vitamin A and D disorders (both are linked to Ca uptake). It is reported that the normal value for Ca in human serum is $92 \pm 7.3\,\mathrm{ug\,g^{-1}}$ [7] in healthy people (normal renal function and nutritional status).

IMEP 17 (International Measurement Evaluation Programme—Trace and minor constituents in human serum) was an international interlaboratory comparison, which assessed state-of-the-practice Ca measurements from 983 field labs in 35 countries. Selection of Ca as an analyte of

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interest was based on recommendations from the C-AQ IFCC (Committee for the Analytical Quality of the International Federation for Clinical Chemistry and Laboratory Medicine). Results showed a spread of data ranging from 59–106% deviation from the reference value for the sample [8]. It was shown that the combined standard uncertainty of routine measurements is ∼3%.

There is a need to have methods that can accurately determine total Ca in serum with a low-uncertainty, to aid medical diagnosis. These methods can then be used to produce high-quality certified reference materials for the clinical analytical community. Multi-elemental analysis is appropriate when offering a high-quality analytical service; however, for the ultimate accuracy required to produce a high-quality certified reference materials single element, IDMS analysis is important.

In complex matrices such as human serum, Ca analysis is complicated by the existence of interferences and the ubiquitous nature of Ca makes sample preparation and blank contamination major analytical issues. The aims of this study were to produce a high-accuracy isotope dilution mass spectrometry (IDMS) reference value for Ca analysis in human serum by a novel octopole collision cell ICP-MS method and comparison of that technique with double-focusing sector field ICP-MS and non-IDMS ICP-OES analysis. Such comparisons embrace state-of-the art octopole collision cell technology and sector field instrumentation in contrast to the optical detection system employed by ICP-OES. Agreement between three different techniques can only serve to increase confidence in certified reference material values since it is very unlikely that the two different (MS and OES) detection systems will suffer from the same interferences, to the same degree. Such confirmatory supporting data from a second instrument allows us to demonstrate the capability to produce high-accuracy certified reference materials at a suitably low-uncertainty.

## 2. Method

### 2.1. IDMS calibrations

High-accuracy analysis by IDMS is now well established [9]. The isotope dilution method utilised for this work here is a modified version of the matching technique originally proposed by Henrion [10] and developed by Catterick and co-workers [11,12]. The technique involves the addition of an isotopically enriched analogue (often referred to as the spike) of the element of interest, which is used as an internal standard. The double-matched IDMS equation as used in this investigation is given in Eq. (1).

$$c'_{\mathrm{x}} = h.c_{\mathrm{z}} \cdot \frac{m_{\mathrm{Y}}}{m_{\mathrm{X}}} \cdot \frac{m_{\mathrm{Zc}}}{m_{\mathrm{Yc}}} \cdot \frac{(R_{\mathrm{Y}} - R'_{\mathrm{B}}) \cdot R_{\mathrm{Bc}}/R'_{\mathrm{Bc}}}{R'_{\mathrm{B}} \cdot R_{\mathrm{Bc}}/R'_{\mathrm{Bc}} - R_{\mathrm{X}}} \cdot \frac{R_{\mathrm{Bc}} - R_{\mathrm{X}}}{R_{\mathrm{Y}} - R_{\mathrm{Bc}}} \quad (1)$$

where $c'_{\mathrm{x}}$ is the mass fraction of analyte in sample X obtained from one measurement; $C_{\mathrm{Z}}$ is the mass fraction of analyte in primary standard Z; $m_{\mathrm{Y}}$ is the mass of spike Y added to sample X to prepare the blend B (X + Y); $m_{\mathrm{X}}$ is the mass of sample X added to the spike Y to prepare the blend B (X + Y); $m_{\mathrm{Zc}}$ is the mass of primary standard solution Z added to the spike Y to make the calibration blend Bc (Bc = Y + Z); $m_{\mathrm{Yc}}$ is the mass of spike Y added to the primary standard solution Z to make the calibration blend Bc; $R'_{\mathrm{B}}$ is the measured isotope amount ratio of the sample blend (X + Y); $R'_{\mathrm{Bc}}$ is the measured isotope amount ratio of the calibration blend Bc; $R_{\mathrm{Bc}}$ is the gravimetric value of the isotope amount ratio of the calibration blend; $R_{\mathrm{Z}}$ is the isotope amount ratio of primary standard Z (IUPAC value [14]) and $h$ is the moisture correction. The use of double IDMS means that we bracket the sample blend B (which contains the sample X, mass $m_{\mathrm{x}}$, with the spike Y, mass $m_{\mathrm{y}}$) with a calibration blend Bc (which is prepared by mixing a gravimetric standard Z, mass $m_{\mathrm{Zc}}$, with the spike Y, mass $m_{\mathrm{yc}}$). Each measured isotope $R'_{\mathrm{B}}$ is therefore corrected by the isotope amount ratio $R'_{\mathrm{Bc}}$ calculated from the average of the isotope amount ratios of blend Bc measured before and after the sample blend B, a process referred to as bracketing. The matching approach of Catterick [11] was employed with an approximate match made between the spiked calibration blend and the spiked sample blend, which resulted in matching counts per second and isotope ratios to within 5%.

### 2.2. ICP-OES exact matching bracketing calibration

IDMS analysis cannot be used for ICP-OES analysis because wavelengths are measured rather than masses. Therefore, samples were analysed by ICP-OES using the exact matching bracketing technique described in detail elsewhere [14]. The technique uses a single elemental standard that is prepared to match the analyte concentration (within 5%). A suitable internal standard is then added to both sample and standards to produce a blend. The intensity of Ca internal standard is also matched to give equal signal intensity. The matching of signal intensities between samples and standards, and analyte and internal standard, reduces the uncertainty associated with instrument linearity. The concentration of calcium in the sample was determined according to Eq. (2):

$$C_{\mathrm{smp}} = h\left(\frac{I_{\mathrm{smp}}/I_{\mathrm{I.S.}}}{I_{\mathrm{std}}/I_{\mathrm{I.S.}}}\right) \times C_{\mathrm{std}} \times D \quad (2)$$

where $C_{\mathrm{smp}}$ is the amount content of Ca in the serum sample, $I_{\mathrm{smp}}$ signal intensity of Ca, $I_{\mathrm{I.S.}}$ signal intensity of internal standard, $I_{\mathrm{std}}$ signal intensity of the gravimetrically prepared calibration standard, $C_{\mathrm{std}}$ concentration of the gravimetrically prepared calibration standard, $D$ the dilution factor prior to analysis and $h$ the moisture correction. The sample was bracketed 10 times with the standard blend for each analytical measurement. The sequence therefore consisted of: blank, mass

Table 1
Abundance of Ca isotopes and the likely interferences

| Ca isotope | Natural abundance (%) [13] | Likely interferences |
|---|---|---|
| 40 | 96.941 | $^{40}Ar^+$, $^{24}Mg^{16}O^+$, $^{39}K^1H^+$ |
| 42 | 0.647 | $^{26}Mg^{16}O^+$, $^{84}Sr^{2+}$, $^{40}Ar^1H_2{}^+$, $^{40}Ca^1H_2{}^+$ |
| 43 | 0.135 | $^{27}Al^{16}O^+$, $^{86}Sr^{2+}$, $^{43}Ca^1H^+$ |
| 44 | 2.086 | $^{26}Mg^{18}O^+$, $^{27}Al^{17}O^+$, $^{28}Si^{16}O^+$, $^{88}Sr^{2+}$, $^{43}Ca^1H^+$ |
| 46 | 0.004 | $^{30}Si^{16}O^+$, $^{92}Mo^{2+}$ |
| 48 | 0.187 | $^{24}Mg^{24}Mg^+$, $^{96}Mo^{2+}$, $^{32}S^{16}O^+$ |

bias standard blend, sample, mass bias standard blend, sample, etc.

### 2.3. Ca mass spectral interferences

The abundance of the Ca isotopes and some of the interferences they are subject to are shown in Table 1. Under ideal circumstances analysis of Ca is achieved by reference to the dominant isotope $^{40}Ca$. However, in standard ICP-MS analysis mode $^{40}Ca$ is difficult to completely resolve from the interfering ion $^{40}Ar^+$, and consequently minor isotopes have to be analysed. This situation was deemed to be beneficial for this study, since this introduces the advantage that samples require fewer sample dilution steps to produce an ion signal that is within the linear range of the instrument detectors. Calcium is an abundant element and prone to laboratory contamination. Hence, there is an advantage in keeping the sample preparation steps to a minimum to reduce the risk of contamination. Additionally, $^{40}Ar^+$ cannot be completely resolved by HR-ICP-MS, and since a direct comparison was required for this study, the isotope pair used for this work was $^{44}Ca/^{42}Ca$ (natural/spike). The exact theoretical optimum for reducing the error propagation factor (EPFopt) [12] was calculated to be 0.2; however, the exact matching IDMS procedure normally aims for isotope amount ratios, which are close to unity. The value of 0.6 was selected as a compromise between unity and the error propagation factor of 0.2; spiking at 0.6 also uses less of the expensive enriched spike solution.

### 2.4. Collision and reaction cells (CC-ICP-MS)

A number of different collision/reaction cells are currently available (ELAN DRC, Perkin Elmer; X-series CCT, Thermo Elemental; Platform ICP, Micromass and 7500c, Agilent Technologies) each employing various combinations of reaction/collision technology. For example, the DRC approach [15] to $Ca^+$ interferences is to use $NH_3$ as a reaction gas and effect a charge transfer reaction of the type $Ar^+ + NH_3 \rightarrow NH_3{}^+ + Ar$ allowing interference-free analysis at $^{40}Ca^+$. Hexapole collision cell instruments have been used for Ca analysis using a mixture of He and $H_2$ reaction gas [16,17]. The approach used in this study was to use an Agilent 7500c (Agilent Technologies, Cheadle, UK) equipped with an octopole reaction system (ORS). The instrument has been described in detail elsewhere [18]. The octopole collision cell instrument utilised $H_2$ cell gas to remove a number of the polyatomic interferences that interfere with the spike isotope $^{42}Ca$ and reference isotope $^{44}Ca$. The advantages of using a simple light reaction gas such as $H_2$ are that scattering losses are kept at a minimum; almost no new interferences are generated and post-cell energy discrimination can be set to filter out any unwanted cell-produced reaction product ions. A simple energy discrimination filter, set up to effect a positive voltage barrier is achieved through manipulation of the dc offset voltage of the collision cell octopole relative to the quadrupole mass analyser, enabling only the higher kinetic energy analyte ions to pass through the quadrupole to be detected. The reaction process described effectively attenuates the polyatomic ion intensity more than the analyte ions. It has been reported [19], after work with a plasma source ion trap (PSIT) mass spectrometer modified with a collision cell, that $H_2$ does not react appreciably with a number of atomic ions including $Ca^+$.

Bandura et al. [20], were the first to report the improved precision achievable for isotope ratio measurements using a dynamic reaction cell. These authors demonstrated that the presence of a reaction/collision gas could increase the residence time of ions in a cell, which would in turn serve to dampen small-scale fluctuations in the ion beam, when compared with non-pressurised cells, an effect commonly known as collisional damping.

On the octopole collision cell the initial ion spread is minimised due to the ShieldTorch System [21] (a grounded metal plate), which is employed to obtain a low initial ion energy and to reduce the ion energy distribution in the ion beam [22]; similar forms of torch bonnet or sheath have also been shown to reduce isotope ratio precision [23]. Use of a collision/reaction cell could not only give accurate interference-free analysis but could also improve isotope ratio precision and therefore reduce the overall uncertainty of the analysis by reducing the contribution from the instrumental precision. For each analytical measurement the sample was bracketed once with the mass bias standard blend. The sample introduction system comprised a PFA microflow nebuliser (Agilent Technologies, Cheadle, UK) with a double pass spray chamber cooled to 2 °C and the torch was fitted with the Agilent ShieldTorch System (STS). The $H_2$ (BOC Gases, Guildford, UK, research grade 99.9995% purity) gas flow rate was optimised for both $^{44}Ca$ and $^{42}Ca$ in the matrix. For Agilent 7500c instrument operating conditions, see Table 2. The ratio $R_{Bc}/R'_{Bc}$ is applied to calculate a mass bias correction factor. In practice, this is obtained by analysing the calibration blend solution before and after analysis of the sample in a bracketing procedure.

### 2.5. Double-focusing sector field ICP-MS

Quadrupole-based instruments have sufficient resolution to separate single mass units but not the much smaller

Table 2
Instrument operating parameters

| Parameter | HR-ICP-MS | Parameter | ICP-OES | Parameter | CC-ICP-MS |
|---|---|---|---|---|---|
| Power W | 1200 W | Power W | 1300 W | Power W | 1500 W |
| Cool gas | 15 L min$^{-1}$ | Cool gas | 15 L min$^{-1}$ | Cool gas | 15 L min$^{-1}$ |
| Auxiliary gas | 0.9 L min$^{-1}$ | Auxiliary gas | 0.5 L min$^{-1}$ | Auxiliary gas | 0.9 L min$^{-1}$ |
| Nebuliser gas | 1 L min$^{-1}$ | Nebuliser gas | 0.8 L min$^{-1}$ | Carrier gas | 1.01 L min$^{-1}$ |
| Runs | 10 | Replicates | 8 | Replicates | 10 |
| Points per peak | 20 | Points per peak | 5 | Points per peak | 3 |
| Sample time | 0.005 s | Integration time | 0.1 s | Integration time | 3.0 s/peak |
| Passes | 100 | Read time | 10 s | Octapole bias | −19 V |
| Resolution | 4500 | Resolution | Normal | Quadrupole bias | −16 V |
| Acquisition window | 120% | Measurement mode | Peak area | Cell gas ($H_2$) flow rate | 4.0 mL min$^{-1}$ |

mass differences which polyatomic interferences require. Utilisation of high-resolution mass spectrometry enables us to resolve out the interferences on $^{44}$Ca and $^{42}$Ca and has been applied to biological sample analysis [24,25]. The most significant interferences are the $^{28}Si^{16}O^+/^{44}Ca^+$, $^{40}ArH_2^+/^{42}Ca^+$ and $^{26}Mg^{16}O^+/^{42}Ca^+$ requiring resolution of 2687, 2349 and 2221, respectively (applying the 10% valley definition); thus, the medium resolution instrument setting of ∼4500 was sufficient to separate out most of the potential interferences on the Ca isotopes. The double-focusing sector field ICP-MS instrument used was the Thermo Finnigan Element 1 (Thermo Electron, Bremen, Germany). Medium resolution of 4500 was employed. A PFA micromist nebuliser (Glass expansion, Melbourne Australia) and double-pass spray chamber cooled to 5 °C was used and the guard electrode were activated. For full operating conditions, see Table 2. For each analytical measurement the sample was bracketed once with the mass bias standard blend to calculate a mass bias correction factor.

## 3. ICP-OES

The ICP-OES instrument was a Perkin-Elmer Optima 3300RL (Perkin-Elmer, Seer Green, UK) with a cross-flow nebuliser and Scott Spray chamber. Scandium was selected as the internal standard and measured at a wavelength of 361.385 nm, and calcium was measured at 393.366 nm. There are no major interferences for any of the Ca wavelengths and the selection of 393.366 nm was based on it giving the optimum detector response for the sample concentration being analysed. The internal standard was added to the blank and the final signal intensities of the sample were corrected relative to the signal intensity of the internal standard in the calibration solution. For full instrument operating conditions, see Table 2.

### 3.1. Samples and sample preparation

Two serum materials were used to test the method performance in this study: a NIST SRM and a blind inter-laboratory comparison sample. NIST SRM909b is a freeze-dried human serum available at two different analyte concentration levels. The CRM is certified for Ca (level 1) at 86.698 ± 0.618 μg g$^{-1}$ when reconstituted according to the instructions on the certificate and density corrected.

LGC as a function of its role as the UK chemical national metrology institute (NMI) also participated in a Comité Consultatif pour la Quantité de Matière (CCQM) study. The study, CCQM-K14 was a key comparison study on the calcium content in human serum. The aim of the study was to assess the current analytical measurement capabilities of a clinical analyte in human serum. The sample was a frozen human serum.

Results will be presented for five replicate samples of NIST 909b level 1 CRM on all three instruments. In addition the LGC result for CCQM-K14 analysed by collision cell ICP-MS will be presented alongside the results from all participants in the study (all of which were NMIs or invited expert laboratories), with full uncertainty budgets.

The same sample preparation was applied to all analyses. Preliminary experiments based on microwave and heating block acid digestion with hydrogen peroxide proved prone to variable blanks caused by contamination problems. Blank levels ranged from 0.8 to 8.3% of the total sample signal with sample digestion. Experiments with Triton-X as a diluent provided blanks ranging from 1.0 to 1.7% of the sample signal. Dilution of the serum with 1% $HNO_3$ generated much more stable and low blank signals ranging from 0.27 to 0.44% of the sample signal. Therefore, the following sample preparation procedure was adopted: the sample (0.5 g) was blended with the spike solution (or internal standard solution for the ICP-OES) and diluted with 1% $HNO_3$ to a total mass of 10–20 g; no other sample manipulation was performed.

### 3.2. Reagents

High-purity acids were obtained from Romil (UK) and used throughout the procedure. Dilution water was obtained directly from an Elga water purification system (18.2 mΩ purity, Marlow, UK). A $^{42}$Ca spike was purchased as $CaCO_3$ from Oak Ridge laboratories, Tennessee, USA and diluted in 10% $HNO_3$ to 3.1 μg g$^{-1}$. The spike enrichment was certified at 93.5% $^{42}$Ca. Two independent sources (Alfa Spec pure Spectra 99.99% $CaCO_3$, dried before use and NIST SRM 3109a 10.397 ± 0.019 mg g$^{-1}$ aqueous Ca solution) of analyte ions were obtained and used to

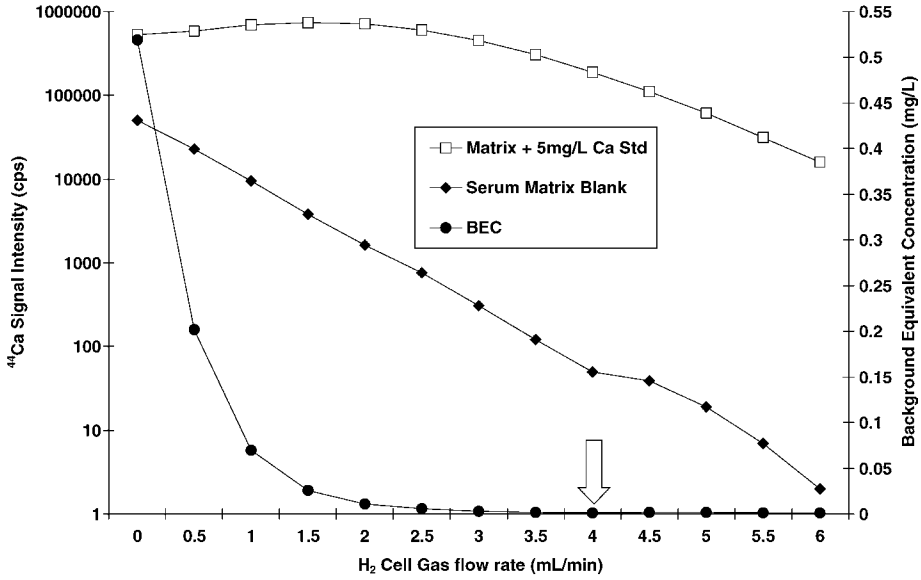


Fig. 1. $H_2$ Cell gas flow rate optimisation graph for the octopole collision cell ICP-MS.

prepare aqueous standards for use as mass bias calibration blends.

## 4. Results and discussion

Calcium isotopic composition is partly governed by radiogenic decay of $^{40}$K [26]. Furthermore, Ca isotopes can fractionate both during natural processes and during the production of high-purity Ca standards. Samples were measured for $^{44}$Ca/$^{42}$Ca ratio relative to the calibration standard solution. No differences could be observed between the ratios of these solutions.

The $H_2$ gas flow rate was optimised on the octopole collision cell for both $^{44}$Ca and $^{42}$Ca in a solution prepared to represent the likely interferences in a serum matrix. The matrix blank was composed of Si and Mg at 10 mg L$^{-1}$, Sr and Al at 10 ug L$^{-1}$ in a matrix of 2% $HNO_3$, 2% HCl and 2% propanol. Si, Mg, Sr and Al were added at levels approximately representative of human serum [27]. This matrix solution was diluted 1:20 and was spiked with 5 mg L$^{-1}$ Ca to generate the analyte intensity profile. The optimisation graph for $^{44}$Ca is shown in Fig. 1, plotting the background equivalent concentration (BEC) against $^{44}$Ca signal intensity. The optimum gas flow rate was 4 mL min$^{-1}$. This was selected to give the best compromise between interference removal and analyte signal intensity. The optimum flow rate for $^{42}$Ca was identical at 4 mL min$^{-1}$.

### 4.1. Uncertainty

The main sources of uncertainty for the IDMS-ICP-MS measurements are from the mass fraction of the primary standard $C_Z$ and spike $C_Y$, the masses of sample and spike used to create the sample blend; the isotope amount ratio of the mass bias blend $R_{Bc}$, the measured isotope amount ratios of the sample and standard blends $R'_B$ and $R'_{Bc}$, and the variation of replicate analyses. The mass fraction of the primary standard ($C_z$) and the associated standard uncertainty were calculated from the data for its gravimetric preparation. The concentration of the spike ($C_y$) was determined by reverse isotope dilution using $C_Z$ as the calibrant. All weighings were performed on a four-figure balance. The standard uncertainty on each mass was determined from the repeatability of calibrated weight measurements and the balance certificate. The isotope amount ratio of the calibration blend is calculated from the data of the spike and the standard solutions that are gravimetrically mixed according to Eq. (3).

$$R_{Bc} = \frac{Rmc\sum R_{Yi} + R_Y m_{Yc} c_Y \sum R_{Zi}}{mc\sum R + mc\sum R} \quad (3)$$

Where $R_{Yi}$ is the isotope ratio of the spike, and all other components are as defined in the equation.

The measured isotope amount ratios ($R'_B$ and $R'_{Bc}$) each comprise 10 individual replicate measurements from which a mean isotope ratio was calculated. The raw data from these measurements was blank subtracted using a reagent blank. The standard uncertainties of the measured isotope ratios were calculated as the standard deviation from the 10 repeated measurements. The variation of replicate analyses was estimated by using the standard deviation of the mean of the five replicate analyses. The uncertainty for each individual measurement is estimated by combining the uncertainties for each of the components in Eq. (1) in accordance with GUM [28].

The final standard uncertainty value was calculated by combining the standard deviation of the mean of five independent replicate analyses with the average uncertainty estimate of individually determinations. This value was expanded

Table 3
Results for CRM NIST 909b Level 1

| Sample | Ca concentration ($\mu g\,g^{-1}$) | Expanded standard uncertainty | Recovery | Relative expanded standard uncertainty |
|---|---|---|---|---|
| NIST 909b Level 1 CRM | 86.98 | 0.62 | | 0.7% |
| High Resolution ICP-MS | 88.08 | 2.06 | 101.3% | 2.3% |
| Collision cell ICP-MS | 86.60 | 0.98 | 99.6% | 1.1% |
| ICP-OES | 87.39 | 1.02 | 100.5% | 1.2% |

to the 95% confidence limit using the coverage factor $k = 2$.

For the ICP-OES data the uncertainty was calculated in a virtually identical way. Differing only in that the samples were bracketed 10 times with the internal standard matched standard the standard uncertainties of the ratios were calculated as the standard deviation of the 10 bracketed ratios, rather than from a number of replicates of one bracketing sequence. The variation of replicate analyses was estimated by using the standard deviation of the mean of the five replicate analyses in the same way as for the octopole collision cell and double-focusing sector field ICP-MS data.

### 4.2. CRM results

The results for NIST 909b CRM on the HR-ICP-MS, the ICP-OES and the CC-ICP-MS are given in Table 3. Results show that all three techniques give excellent agreement with the certified values. The instrument giving the best recovery of the CRM is the CC-ICP-MS with $H_2$ reaction gas, this method also gives the lowest relative expanded standard uncertainty. The ICP-OES also gives a very good recovery and comparable expanded standard uncertainty, which is very encouraging given the two totally different analysis techniques. The two techniques also represent total independence with regard to potential spectral interferences. If the collision cell instrument is run in standard mode (i.e. no reaction gas or cool plasma conditions [29]) the resulting recovery of the CRM is 2.8% too high indicating the additive effect of the interferences present and demonstrating the interference removal success of the collision cell system for Ca analysis. The higher total expanded uncertainty for the double-focusing sector field ICP-MS can be explained by the lower precision of the isotope ratios than measured by octopole collision cell ICP-MS, the mean R.S.D. of the $^{44}Ca/^{42}Ca$ ratio for double-focusing sector field ICP-MS was 1.5%, and for the octopole collision cell was, 0.4%. The reasons for worse precision being observed by HR-ICP-MS are not counting statistic dependant, since the sensitivity is comparable/greater on the HR-ICP-MS compared to CC-ICP-MS. Worse precision could however be expected at medium resolution than at low resolution because medium resolution does not result in flat-topped peaks. The HR-ICP-MS is also an older instrument than the CC-ICP-MS and the plasma is much less robust therefore much more noisy, resulting in lower precision isotope ratios.

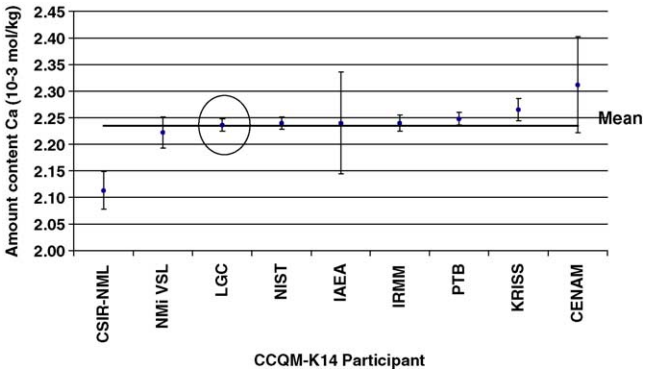


Fig. 2. The results for all participants in CCQM-K14 Ca in human serum.

### 4.3. CCQM results

LGC submitted results for CCQM-K14 from collision cell data. This laboratory had previously submitted ICP-OES results for a pilot study (CCQM-P14) on the same matrix, which were ~2% lower than the mean result. This slightly low result was examined and ascribed to the combined effects of matrix-induced interferences affecting performance of the internal standard and measurement of the analytical blank [30]. For this study the addition of the internal standard to the blank and correction of signal intensities accordingly had solved the problems of blank contribution. The results from the CCQM-K14 serum study that LGC participated in are plotted in Fig. 2 [31].

The chart clearly shows that the result obtained ($2.236 \times 10^{-3}$ mol $kg^{-1}$) shows the best agreement with the mean result of all participants ($2.235 \times 10^{-3}$ mol $kg^{-1}$), with one of the lowest relative expanded standard uncertainties reported in the study.

## 5. Conclusions

Results for all three instruments demonstrate accuracy ranging from 99.6% ± 1.1% to 101.3% ± 2.3%. Results show that when compared with double-focusing sector field ICP-MS and ICP-OES, octopole collision cell ICP-MS using $H_2$ reaction gas produces the most accurate (99.6% ± 1.1%) results for analysis of Ca in human serum CRM NIST 909b Level 1 and with the lowest total expanded uncertainty of the three techniques. Additionally, the result obtained by ICP-OES demonstrates excellent agreement with only

a marginally larger uncertainty than octopole collision cell ICP-MS suggesting that accurate analysis of Ca in a serum matrix can also be carried out using a non-IDMS methodology with an ICP-OES instrument. This equivalence between the two techniques is possible because of the relatively high levels of Ca, it is anticipated that for lower Ca trace levels the sensitivity of ICP-MS would result in much better data. Results that were submitted for a recent CCQM international comparison study based upon the octopole collision cell ICP-MS methodology demonstrated excellent agreement with the mean result of all participants, with a small total expanded uncertainty budget. This study has demonstrated a high-accuracy (99.6%) low-total expanded uncertainty (∼1%) isotope dilution mass spectrometry (IDMS) reference value for Ca analysis in human serum by a novel octopole collision cell ICP-MS method. The analytical results from this study can now be used to underpin the production of certified reference materials for clinical analysis of human serum.

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