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IZiNCG TECHNICAL BRIEF

Comparison of laboratory instrument types for analysis of plasma or serum zinc concentration

The importance of plasma or serum zinc concentration, and how it is measured

Plasma or serum zinc concentration (PZC) is considered the best available biomarker of individual and population zinc status (1). When more than 20% of individuals in a population has a PZC below the relevant cutoff — accounting for age, sex, fasting status, time of day and pregnancy status — the risk of zinc deficiency is considered an issue of public health importance (2).

Although the prevalence of low PZC is associated with the prevalence of inadequate zinc intake, numerous other physiological and methodological factors may also influence PZC (1). IZiNCG Technical Briefs Number 2 and Number 6, and IZiNCG's Practical Tips documents provide more information on these issues (2-4). However, the potential effects of other analytical issues, such as the type of laboratory instrument used, have not been systematically evaluated.

Zinc analytical instruments

Three instrument types are commonly used for PZC analysis: atomic absorbance spectrometers (AAS), inductively-coupled plasma optical emission spectrometers (ICP-OES), and ICP mass spectrometers (ICP-MS). Sampling and calibration are similar for all three types of instruments. However, the instruments differ in how samples are energized, the type of emission measured, sensitivity, and operational cost (**Table 1**).

Table 1: Similarities and differences between instrument types used for zinc analysis

	AAS	ICP-OES	ICP-MS
Sample preparation	Samples prepared in solution are fed into the instrument, energized, and the zinc content is measured based on emission or energy output		
What energizes the sample	Flame or furnace	Plasma (hotter and more uniform than flame or furnace) generated by induction (using a magnetic field)	
What is measured	Optical emission (brightness) at the wavelength (color) for zinc		Electrical emission of one or more zinc isotopes as they transition between ionized states in plasma
Calibration method	Zinc standard solution, at several known concentrations (calibrators), covering the range of likely zinc concentrations for the sample (i.e. standard curve calibration)		
Sensitivity	Lowest	Intermediate	Highest
Complexity and cost of operation	Lowest	Intermediate	Highest

IZiNCG Laboratory Methods Study

IZiNCG designed a laboratory methods study to assess the accuracy and precision of different laboratory instruments used for analyzing zinc concentrations in plasma and serum. The specific objectives of the study were to:

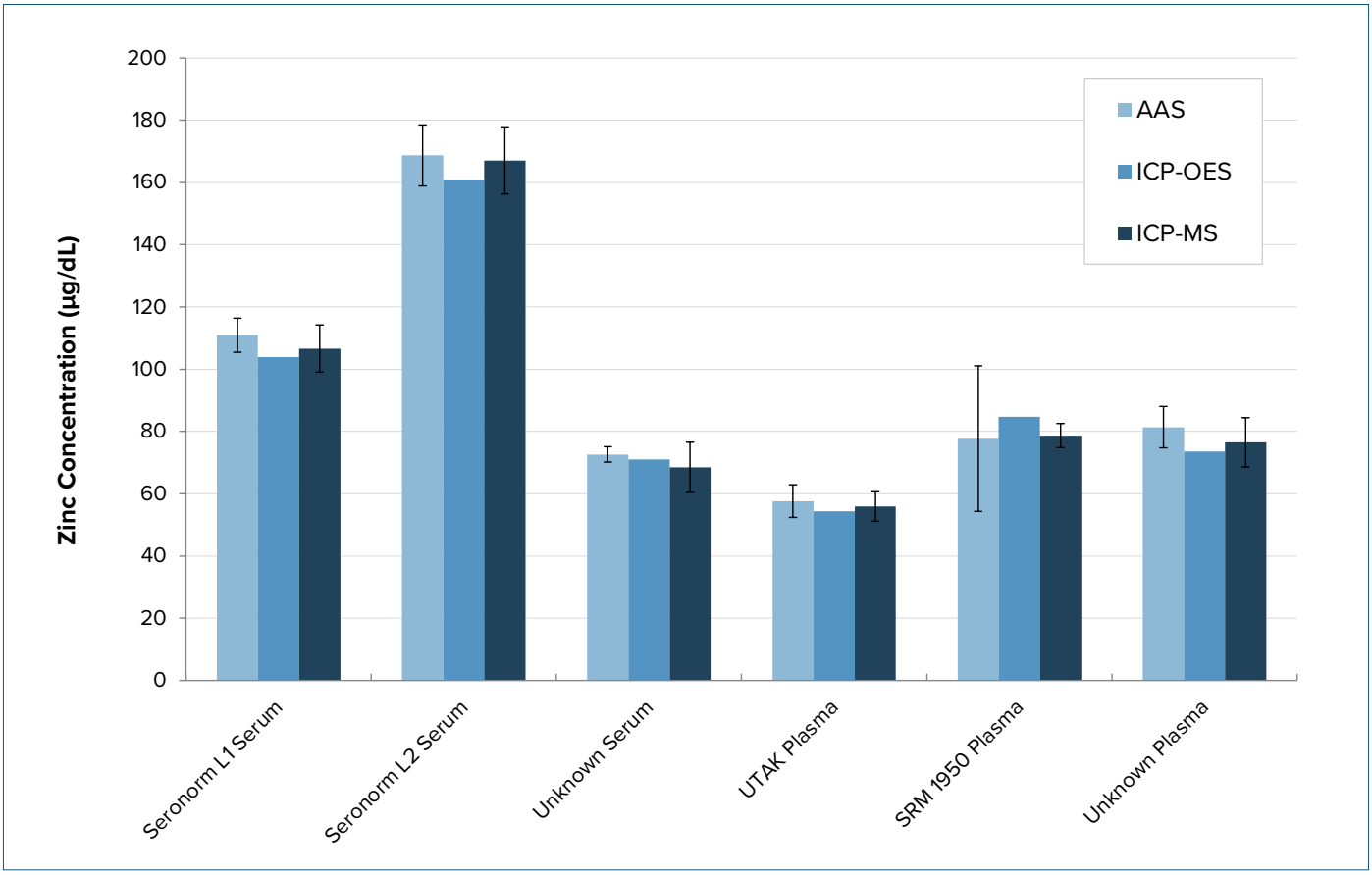
1. Compare the analytical accuracy and precision among the three different instrument types;
2. Compare the analytical accuracy and precision among serum and plasma.

Powdered liver (used to measure zinc content in food composites) was also included in the methods study but the results were not included for the purpose of this brief.

Summary of the methods

A reference method for zinc analysis was first developed (5). Then, multiple laboratories in low-, middle- and high-income countries were invited to participate in the activity. Each laboratory received a standard set of samples, materials and reagents, and reference methods for sample preparation and analysis. These included trace element grade nitric acid, ultrapure water, filter pipette tips, National Institute of Standards and Technology (NIST) SRM 3168a zinc solution for instrument calibration, a pre-diluted zinc standard solution for checking calibration, and reference and unknown serum and plasma samples. Laboratories were requested to run the zinc laboratory analyses and share their results with IZiNCG.

Figure 1. Zinc concentration of reference and unknown serum and plasma, by instrument type



Zinc concentration of each serum and plasma sample was determined by AAS (n = 4), ICP-OES (n = 1), and ICP-MS (n = 4). Data are displayed as mean ± standard deviation. Standard deviation for ICP-OES is not calculated since only one such instrument was used. No differences were detected between AAS and ICP-MS for any material, or overall.

Accuracy was evaluated using percent error: the difference between the laboratory measured value and the known zinc value of each reference sample. Precision was evaluated using the coefficient of variation (CV) of repeated zinc concentration measures for each sample analyzed: the spread or variation of measured values relative to the mean value.

Results

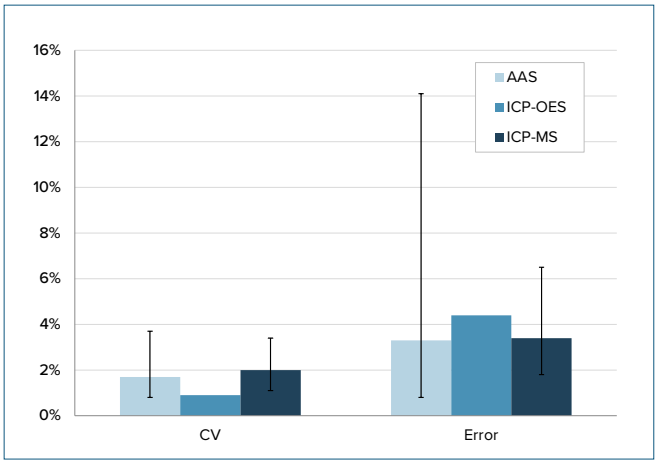
Seven laboratories in four countries, including two low- to middle-income countries, using nine instruments, participated in the study. Four AAS, one ICP-OES, and four ICP-MS instruments were used. No differences in zinc concentration due to type of instrument (ICP-MS, ICP-OES, AAS) were observed (**Figure 1**). Likewise, there were no differences in the precision or accuracy of the analyses in relation to the type of laboratory instrument (**Figure 2**).

The same zinc reference (NIST SRM 1368a) was used by each lab to calibrate its instruments. Although the intent of the study was not to compare individual laboratories, our analysis revealed differences in instrument calibration were as high as 15% between individual instruments (**Figure 3**). Such differences in calibration could lead to large differences in the proportion of a population falling below a cutoff for zinc deficiency.

One AAS instrument also drifted, where the measured value of the diluted zinc standard changed by more than 5% over the course of the analytical run (data not shown). The observation of drift illustrates the need to monitor each instrument, and verify that calibration was maintained over the course of analysis.

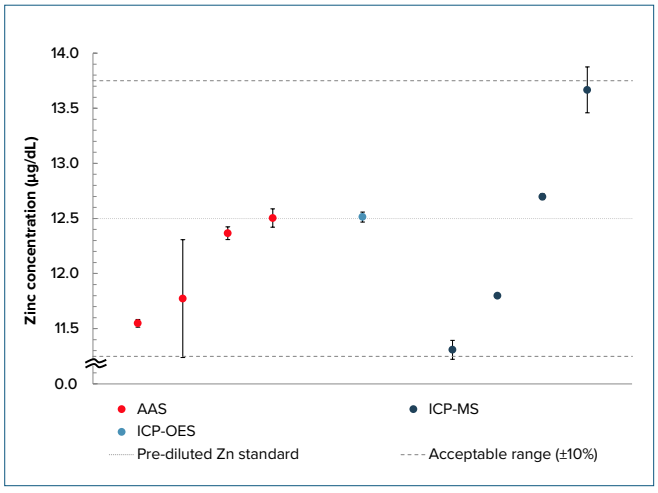
Despite such differences in calibration, the data provided no indication that instrument choice affects the accuracy or precision of PZC measurement when the same materials and methods are employed. No statistically significant differences in precision or accuracy were observed due to the type of instrument used (**Figure 2**).

Figure 2: Precision and Accuracy



The overall CV (for precision) and error (for accuracy) of each instrument were determined from all samples analyzed by that instrument. The geometric mean (95% confidence interval) of the CV was 1.7% (1.2%, 2.4%) overall; 1.7% (0.8%, 3.7%) for AAS, 0.9% for ICP-OES, and 2.0% (1.1%, 3.4%) for ICP-MS. The error was 3.5% (2.2%, 5.6%) overall; 3.3% (0.8%, 14.1%) for AAS, 4.4% for ICP-OES, and 3.4% (1.8%, 6.5%) for ICP-MS. No differences in the CV ($p = 0.61$) or error ($p = 0.95$) were detected between AAS and ICP-MS ($n = 4$ each for AAS and ICP-MS). ICP-OES was not included in the comparison because there was only one such instrument.

Figure 3: Calibration



Calibration was verified using a pre-diluted NIST SRM 3158a zinc standard solution with target concentration of 12.5 µg/dL, indicated by a grey dashed line. The zinc solution was analyzed by each instrument (AAS n=4, ICP-OES n=1, and ICP-MS n=4) in triplicate at the beginning of the analytical run. Mean ± standard deviation are displayed for each instrument. The mean zinc concentration was 12.2 ± 0.7 µg/dL overall, and was not significantly different ($p = 0.96$) between AAS (12.2 ± 0.5 µg/dL, $n = 4$) and ICP-MS (12.4 ± 1.0 µg/dL, $n = 4$). Since there was only one ICP-OES instrument (12.5 µg/dL, $n = 1$), it was not included in the statistical comparison.

Summary

When standardized methods are used for the preparation and analysis of zinc concentrations in plasma and serum, each type of instrument (whether AAS, ICP-OES, or ICP-MS) provides similar mean results and similar accuracy and precision.

Suggestions for future efforts

TUNING

The procedures of each individual laboratory can influence the accuracy and precision of zinc analysis results. Tuning refers to the action of refining these procedures for optimal accuracy and precision. Some procedures may be specific to the individual laboratories due to equipment or the type of sample preparation. Therefore the action of verifying procedures at the individual laboratory is important for optimal results. We recommend that laboratories established in zinc analysis, and those interested in getting started in zinc analysis:

1. Review Analytical Methods for Zinc in Human Studies: Plasma, Serum, and Food Composites (5).
2. Establish or expand internal quality assurance procedures for zinc analysis. A useful reference is Quality Assurance Principles for Analytical Laboratories, by AOAC International (6).

HARMONIZATION

To ensure consistent and accurate results across laboratories, there is a need for harmonized practices and standards around quality assurance, laboratory verification, capacity building, distribution of verified controls and reference materials, and tuning and calibration methods.

References

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About IZiNCG

IZiNCG is the International Zinc Nutrition Consultative Group whose primary objectives are to promote and assist efforts to reduce global zinc deficiency through interpretation of nutrition science, dissemination of information, and provision of technical assistance to national governments and international agencies. IZiNCG focuses on identification, prevention and treatment of zinc deficiency in the most vulnerable populations of low-income countries.

IZiNCG Secretariat

University of California San Francisco
5700 Martin Luther King Jr Way
Oakland, CA, USA 94609

secretariat@izincg.org