Biochemical indicators are an objective and quantitative means of assessing the zinc status of a population. They are useful for identifying populations and specific subgroups that are at elevated risk of zinc deficiency and therefore can be used to target interventions to specific high-risk groups.

Serum or plasma zinc concentration is the best available biomarker of risk of zinc deficiency in populations, for a variety of reasons described below. WHO, UNICEF, IAEA, and IZiNCG jointly recommend the use of serum zinc concentration for assessment of population zinc status [1].

For the correct use of serum zinc concentration as an indicator of zinc status, there are several important technical and processing issues that must be considered regarding sample collection, laboratory analysis, and interpretation of the data.

Why use serum zinc concentration as an indicator of zinc status?

Serum zinc concentration has some important characteristics that make it a good indicator of zinc status for populations:

i. it reflects dietary zinc intake;
ii. it responds consistently to zinc supplementation; and
iii. reference data are available for most age and sex groups.

To date, serum zinc is the only biochemical indicator of zinc status known to meet these criteria.

Experimental studies of dietary zinc restriction in adult volunteers have found that the serum zinc concentrations of previously well-nourished individuals decline within a few days or weeks after their zinc intake is severely restricted. Some, but not all, studies of moderately restricted zinc intakes have shown that serum zinc declines, although the response takes longer and is less consistent. Research also shows that serum zinc concentration consistently rises when individuals consume zinc supplements in addition to their usual diet, regardless of their initial serum zinc concentration [2]. Thus, there is strong evidence to indicate that, in general, serum zinc concentration reflects a person’s usual zinc intake during the previous few weeks or months. However, other factors can independently affect the serum zinc concentration. For example, infection can lower serum zinc concentration, while muscle breakdown during weight loss can liberate zinc to the circulation and increase serum zinc concentration.

For these reasons, serum zinc concentration may not be a reliable indicator of an individual’s zinc status. Nevertheless, the distribution of serum zinc concentrations among a representative sample of a population can be used to assess the risk of zinc deficiency in that population. In addition, because the serum zinc concentration rises consistently in response to zinc supplementation, this indicator can be used as evidence of successful implementation of a zinc intervention program [2].

Technical issues concerning the collection, processing, and analysis of specimens for serum zinc concentration

Blood specimens should be taken from the vein of a representative sample of people among populations or subgroups of interest, as defined by age, geographic region, socio-economic status, or other descriptors. More information on sample selection and sample size issues is available in the 1st IZiNCG technical document [3].

If serum zinc concentration is used to assess the impact of a zinc intervention strategy, such as supplementation, fortification or dietary diversification, it is important to schedule the final blood collection before the end of the intervention occurs, because serum zinc concentrations decline to baseline levels within a few days after discontinuing zinc supplementation.

Zinc is present in the serum in very low concentrations, so any contamination from exogenous sources of zinc can dramatically alter the test results. Therefore, samples must be collected and processed using zinc-free needles, syringes or evacuated tubes, centrifuge tubes, storage vials, and transfer pipettes, while avoiding the destruction of red blood cells—hemolysis—and contamination of specimens with ambient zinc in air or water, or by contact with the analyst. A detailed description of appropriate blood collection techniques and suitable materials that can be used for processing the specimens is provided in the 1st IZiNCG technical document [3].

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1 Zinc concentration can be measured in either blood serum or plasma; for the sake of simplicity this document uses the term “serum zinc” or “serum zinc concentration” to refer to both serum and plasma specimens.
Ideally, specimens should be collected according to a strict protocol that controls the time of day and fasting status of the specimen donor. Because it may not always be possible to collect specimens at the same time of day from all subjects, the time of the blood drawing should be recorded, so the resulting values can be adjusted statistically as necessary. Likewise, it is not always possible to ensure that all subjects have either fasted or eaten within a defined time period (for children), the time of the previous meal also should be noted. Once the blood is obtained, it should be stored in a cool box or in a refrigerator until centrifuged to separate the serum or plasma from the blood cells. This will reduce the introduction of possible artifact into the final results due to transfer of zinc from the blood cells to the serum or plasma. When the storage temperature is 2 – 10 °C, the blood samples are stable for up to 24 hours. If the cold chain cannot be guaranteed until the sample is processed, it is important to separate the serum or plasma from the red blood cells within 20-30 minutes. Following centrifugation, the serum or plasma should then be transferred to a screw-top vial for storage, under refrigeration (for up to several days) or frozen, until analysis.

Zinc concentration can be measured by a number of different analytic instruments, such as flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, and neutron activation analysis. The measurement method depends on the local availability of these instruments and the desired level of precision.

Interpreting the results: reference values and suggested cutoffs for adequate serum zinc concentration

Results of the analyses should be compared with the appropriate reference data for age, sex, time of day, and time since last meal to ensure accurate interpretation of the data. Results should be reported as means, ranges, and percentages below the appropriate reference cutoff values for the population as a whole and for selected sub-groups, as described below.

Reference values for serum zinc concentration are based on results obtained from a large sample of presumably well nourished Americans who participated in the NHANES II survey and were free from infection on the day of the blood sample and not taking any medications that may have affected their results [4]. Because serum zinc concentrations vary by age group, sex, time of day of the blood collection and fasting status of the individual, lower limits of normal (i.e., the 2.5th percentile) are presented separately for each of these categories, as shown in Table 1.

### Table 1: Suggested lower cutoffs for serum zinc concentration (µg/dL)

<table>
<thead>
<tr>
<th>Time of day and fasting status</th>
<th>&lt;10 years</th>
<th>≥10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males and females</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>65</td>
<td>66</td>
</tr>
<tr>
<td>Males</td>
<td>57</td>
<td>59</td>
</tr>
</tbody>
</table>

1 For conversion to μmol/L, divide by 6.54.
2 Fasting is defined as no food or beverage consumption for at least 8 hours.

Ideally, an acute-phase protein indicative of infection or tissue damage should be measured to help interpret the results. C-reactive protein (CRP) and α-1 acid glycoprotein (AGP) are acute-phase proteins that can be used for this purpose. If the concentration of the protein is greater than the normal threshold, it could indicate underlying inflammation, which reduces serum zinc concentration. When elevated acute-phase protein levels are found, the corresponding zinc values can be adjusted statistically or eliminated from the database, although this latter approach may introduce selection bias to the results.

Population assessment should be repeated periodically at the time of general nutritional status surveys to monitor changes in the risk of zinc deficiency and response to intervention programs.

This technical brief was prepared by Dr. Kenneth H. Brown and was reviewed by members of the IZiNCG Steering Committee.

IZINCG recommends that if more than 20% of the population (or population sub-group) has a serum zinc concentration below the relevant cutoff, the whole population (or sub-group) should be considered to be at risk of zinc deficiency [3].
Steps in measuring serum zinc concentration to assess population zinc status

Sample collection
- Clean subject’s skin with alcohol at site of the antecubital vein
- Restrict occlusion of subject’s arm with tourniquet for < 1 minute
- Draw blood using stainless steel needle, and collect into trace element-free evacuated blood collection tubes
- Avoid zinc contamination (see table 2)

Data collection
- Age
- Sex
- Time of day
- Time since last meal
- Presence of symptoms of infection
- Other confounding factors (e.g. use of oral contraceptive agents and other hormones)

Sample preparation
- Place blood sample in refrigerator or over ice in cold box and allow to clot 20-30 min
- Centrifuge blood sample at 2000-3000 × g for 10 minutes and separate serum or plasma
- Discard any obviously hemolyzed samples

Sample storage
- Store serum or plasma samples frozen (or in refrigerator if they are to be analyzed within 1-2 days)

Sample analysis
- Dilute sample for zinc 10-fold in solvents
- Read sample zinc concentration using an available instrument with appropriate standard dilutions, in-house quality controls, and Standard Reference Materials
- Consider the measurement of one or more acute phase protein (CRP, AGP)

Data analysis
- Use appropriate cutoffs depending on characteristics of study population (see table 1)
- Correct for time of day or time since last meal unless sample collection was standardized
- Adjust serum zinc concentration statistically if acute phase protein is elevated
Table 2: Precautions to avoid zinc contamination

- Disposable polyethylene gloves, free of talc or other coatings, worn by those handling blood samples;
- Samples processed in laminar flow clean rooms, laminar flow hoods, or otherwise clean, dust and smoke-free laboratory;
- Stainless steel needles;
- Anti-coagulants (if separating plasma) that are pre-screened and documented to be zinc-free;
- Trace element-free evacuated tubes for blood collection, stoppers, and serum separators; should be pre-screened for zinc prior to use;
- Pre-screened, polyethylene processing and storage vials;
- Except for pre-screened disposable equipment, all equipment used should be decontaminated by washing procedures (soaked for 24 hours in ultrapure 10-20% HCl or HNO₃ solution and rinsed 3-4 times in distilled, deionized water);
- All materials and equipment stored covered or sealed to avoid dust.

References


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. The Steering Committee of IZiNCG consists of 11 well-recognized international scientists with longstanding expertise in zinc nutrition and public health programs.