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Establishing serum zinc reference intervals with two different photometric assays and evaluating their impact on zinc deficiency prevalence

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Abstract

Objectives: The results obtained for zinc levels by different laboratory measurement procedures vary because zinc is not yet a harmonized test. Firstly, we aimed to determine reference intervals for serum zinc that had been measured by two different kits on two different clinical chemistry analyzers, employing various statistical methodologies, and secondly to make a comparison between zinc deficiency prevalences according to the determined reference intervals and the manufacturer's reference intervals.

Methods: The results of the serum zinc levels that were measured by spectrophotometric method, using Improgen® and Archem® zinc kits across a range of time intervals, were obtained retrospectively. The indirect reference intervals for zinc were determined using the Bhattacharya, RefineR and ReflimR methods for both assays. The prevalence of zinc deficiency was evaluated according to the two different kit manufacturers' recommendations and our established reference intervals.

Results: The reference intervals determined by all three methods were found to be lower than those recommended by the manufacturers with the exception of Archem® kit in children. Although the determined reference intervals and lower reference limits were different, the prevalence of zinc deficiency has decreased substantially after the implementation of established reference intervals for both kits and has reached almost same level (20.0–4.6 % and 8.5–4.7 %).

Conclusions: The establishment of appropriate and accurate zinc reference intervals is of paramount importance in order to avoid the overdiagnosis of zinc deficiency, the unnecessary laboratory testing and the administration of supplements to individuals without underlying deficiencies.

Keywords: zinc; zinc deficiency; reference interval; reference limit; cut-off

Introduction

Zinc is an essential mineral that plays a role in many vital functions. It is the most abundant trace element in the body after iron, and is found mainly in skeletal muscles and bones [1]. Zinc plays a role in many parts of metabolic processes. It is found in the structure of many enzymes and is necessary for catalytic activity. It is involved in DNA synthesis and repair, cell division and differentiation, cellular signalling, wound healing, healthy growth and development, and the immune system [2, 3].

Zinc deficiency remains a major public health problem worldwide. Zinc status has been shown to be strongly associated with the economic development of regions, with prevalence exceeding 20 % in most low- and middle-income countries [4]. Deficiency is also seen in developed regions due to chronic disease, age and malabsorption syndromes [4]. Zinc deficiency causes delayed sexual maturation, impotence, hypogonadism, oligospermia, alopecia, dysgeusia (impaired taste), immune dysfunction, night blindness, impaired wound healing, and various skin lesions. Acrodermatitis enteropathica is an autosomal recessive disease in which zinc absorption is impaired. It is characterized by signs and symptoms of severe zinc deficiency including diarrhea, dermatitis (especially perioral and perianal), alopecia, poor growth, and poor immune function [5].

Zinc concentration can be measured using several different analytical methodologies such as inductively coupled plasma mass spectrometry (ICP-MS), spectrophotometry, flame atomic absorption spectrometry (AAS), optical emission spectrophotometry with inductively coupled plasma (ICP-OES). The gold standard method is ICP-MS [6]. The results obtained for zinc levels by different laboratory measurement procedures vary. Zinc is not yet a harmonized test [7].

The International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute

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(CLSI) recommend that each laboratory establish its own reference intervals [8, 9]. Indirect methods estimate reference intervals (RIs) by applying statistical algorithms and data-mining techniques to large-scale patient datasets, aiming to extract the distribution associated with non-pathological cases [9]. Among these, Bhattacharya is a conventional method that has been widely adopted [10]. Recently, two R-based methods, RefineR and ReflimR, have been introduced to facilitate reference interval estimation through automated workflows [11, 12]. Notably, the RefineR algorithm employs an inverse modelling framework, distinguishing itself from the conventional forward modelling strategies adopted by most indirect estimation methods [11].

The first objective of this study was to determine indirect reference intervals for serum zinc, measured by two different kits on two different clinical chemistry analyzers, employing various statistical methodologies. Secondly, the study sought to make a comparison between the reference intervals that had been determined and those that had been recommended by the manufacturers, with the prevalence of zinc deficiency being a key consideration in this regard.

Materials and methods

Data

The serum zinc results, which were measured by two different kits on different analyzers across a range of time intervals in our laboratory, were obtained retrospectively via laboratory information system. The first group of zinc levels consisted of the results between the dates of 01/01/2022–01/01/2023, while the second group of zinc levels consisted of the results between the dates of 07/02/2024–07/07/2024. Results that were rejected due to hemolysis and newborns (0–1 month) due to limited numbers were excluded. In cases where there was more than one zinc result, only the first result was included and results after the first one were excluded. After the exclusions, 42,969 results in the first group and 11,895 results in the second group were evaluated. Ethical approval was obtained from our hospital ethics committee (date:16/07/2024, approval no:155).

Biochemical analysis

The first group of zinc levels measured spectrophotometrically by a commercial kit (Improgen Diagnostics, Istanbul, Turkey) on Roche Cobas c501 analyzer (Roche Diagnostics, Tokyo, Japan) in our laboratory. The inter-assay and intra-assay coefficients of variation of this method were 1.5% and 2.9 %, respectively. The second group of zinc levels were measured spectrophotometrically by a commercial kit (Archem Diagnostics, Istanbul, Turkey) on Beckman Coulter AU480 analyzer (Beckman Coulter, CA, USA) in our laboratory. The inter-assay and intra-assay coefficients of variation of this method were 1.6 % and 3.3 %, respectively.

Statistical analysis

All analyses were performed separately for the two groups measured by different kits on different analyzers. Subsequently, the zinc reference intervals were determined by employing the Bhattacharya, RefineR and ReflimR methods on the entire dataset, without the exclusion of any outliers [10–12].

In accordance with the Bhattacharya method, all data were divided into subgroups in a manner that ensured their distribution was uniform within a specified 'h' class interval. The midpoint of each subgroup was then identified. The natural logarithm (ln) of the frequency (n) of each subgroup was taken. The delta (Δ) ln values were obtained by subtracting the ln value of the subsequent subgroup from that of each preceding subgroup. A scatter plot was constructed displaying Δln values on the y-axis and midpoint of each subgroup on the x-axis. The points in the obtained scatter plot that demonstrated linearity were deemed to be health-related data. The λ value, which represents the point of intersection of x-axis of the linear line was calculated. With the assistance of the λ value, the mean (μ) value of the method was calculated using the formula $\mu=\lambda+h/2$, and the standard deviation value (σ) was calculated with the aid of the equations $\sigma^2=h/slope - h^2/12$. The lower and upper limits of the reference intervals determined by the Bhattacharya method were calculated using the formula $\mu \pm 2\sigma$ [10].

The RefineR and ReflimR methods were applied through the publicly available ReferenceRangeR tool (https://kc.uol.de/referenceranger/) [13].

The necessity of partitioning the zinc reference interval according to gender and manufacturer's age groups (1 month-17 years and ≥18 years) was tested with the Harris-Boyd model [14]. The R value was calculated by employing the ratio of the larger standard deviation value to the smaller value in the subgroups (σ2/σ1). Since all of the R values were less than 1.5, z values were calculated via Harris-Boyd equation [14] and all of the calculated z values were lower than the critical z value of 3, there was no need for partitioning the zinc reference interval according to gender and age. Moreover, the reference limits derived from gender- and age-specific subgroups remained within the bounds of permissible uncertainty, indicating no requirement for stratification of the population data [15].

The prevalence of zinc deficiency was evaluated according to the two different manufacturers' (Improgen® kit's reference intervals were 63–110, 70–114 and 72–127 µg/dL, while Archem® kit's reference intervals were 64–110, 70–114 and 73–127 µg/dL for children, adult females and adult males, respectively) and our established reference intervals. The chi-square test was used for these comparisons. All statistical analyses were performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and SPSS 17 (SPSS Inc., Chicago, Illinois, USA) and $p<0.05$ was considered statistically significant.

Results

The reference intervals recommended in the manufacturers' kit inserts and the indirect reference intervals determined by three different statistical methods in the present study are presented in Table 1.

The reference intervals determined by all three methods were found to be lower than those recommended by the manufacturers with the exception of Archem® kit in children (Table 1). Despite the similarity in the reference intervals recommended by both kit manufacturers, the indirect reference intervals determined by all three statistical methods for the Improgen® kit were observed to be lower than those for the Archem® kit.

Linearity plots of the reference intervals obtained by the Bhattacharya method for both kits are shown in Figure 1.

The distribution of zinc levels and the reference intervals for both kits, as determined by the RefineR and ReflimR methods, were illustrated in Figure 2.

Using the lower reference limits estimated by RefineR for both assay kits, the prevalence of zinc deficiency was calculated (see Figure 3).

Following the implementation of defined reference intervals for the Improgen® kit, the prevalence of zinc deficiency exhibited a notable decline. In children, the percentage decreased from 6.4 to 3.6 %, while in adult males, it decreased from 19.9 to 3.3 %. Similarly, in adult females, the prevalence dropped from 29.3 to 5.6 % ($p<0.001$ for all).

Following the implementation of defined reference intervals for the Archem® kit, the prevalence of zinc deficiency decreased from 8.5 to 3.1 % in adult males and from 11.8 to 6.1 % in adult females ($p<0.001$ for both). On the contrary, in children, the percentage increased from 2.1 to 2.9 % ($p<0.001$).

Discussion

The reference intervals determined for both kits were lower than the reference intervals in the kit inserts with the exception of the Archem® kit in children. This may be due both to the fact that our method of zinc measurement was different from the method used in the studies in which the reference intervals were obtained in the kit inserts and that these studies were conducted in a different population. Furthermore, the reference intervals we determined for the Improgen® kit were lower than the reference intervals of the Archem® kit. While the reference intervals we determined for the two kits were different, after the implementation of defined reference intervals, the prevalence of zinc deficiency was so close in all children and adults as expected in the similar population.

The prevalence of inadequate zinc intake is estimated to be 17 % of the global population, primarily due to limited accessibility to animal-based foods in many populations and the suboptimal status of plant foods as zinc sources, particularly in Sub-Saharan Africa and South Asia [16]. In a study summarising the results of 19 national surveys from low- and middle-income countries, in 13 of 19 surveys prevalence of zinc deficiency were found >20 % in children and 13 of 14 surveys prevalence of zinc deficiency were found >20 % in women [17]. Most of the surveys in the study used The International Zinc Nutrition Consultative Group (IZiNCG) cut-off values, but some countries such as Afghanistan, Azerbaijan, Nigeria, Republic of Maldives, Sri Lanka and China used lower cut-off values and thus found lower prevalence of zinc deficiency in children [17]. Furthermore, the type of specimens (plasma or serum) and measurement methods (ICP-MS, AAS, ICP-OES) used to measure zinc in the

Table 1: Zinc reference intervals (µg/dL) recommended by manufacturers and determined in this study.

	Manufacturer's		This study's							
			Improgen®				Archem®			
	Improgen®	Archem®	n=42,969	RefineR	ReflimR	Bhattacharya	n=11,895	RefineR	ReflimR	Bhattacharya
Children (1 month – 17 years)	63–110	64–110	14,328	60–106	61–105	57–101	4,236	66–113	67–115	64–114
Adult (≥18 years) female	70–114	70–114	21,334				5,836			
Adult (≥18 years) male	72–127	73–127	7,307				1,823			

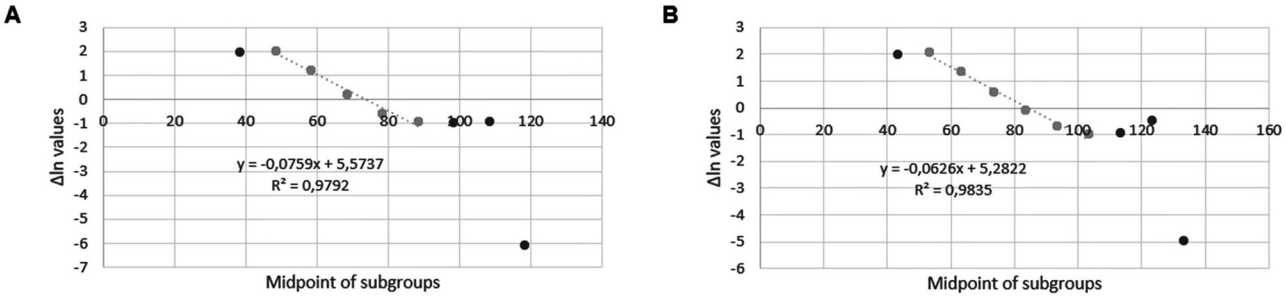

Figure 1: Linearity plots for the reference intervals obtained by the Bhattacharya method. (A) Improgen kit. (B) Archem kit.

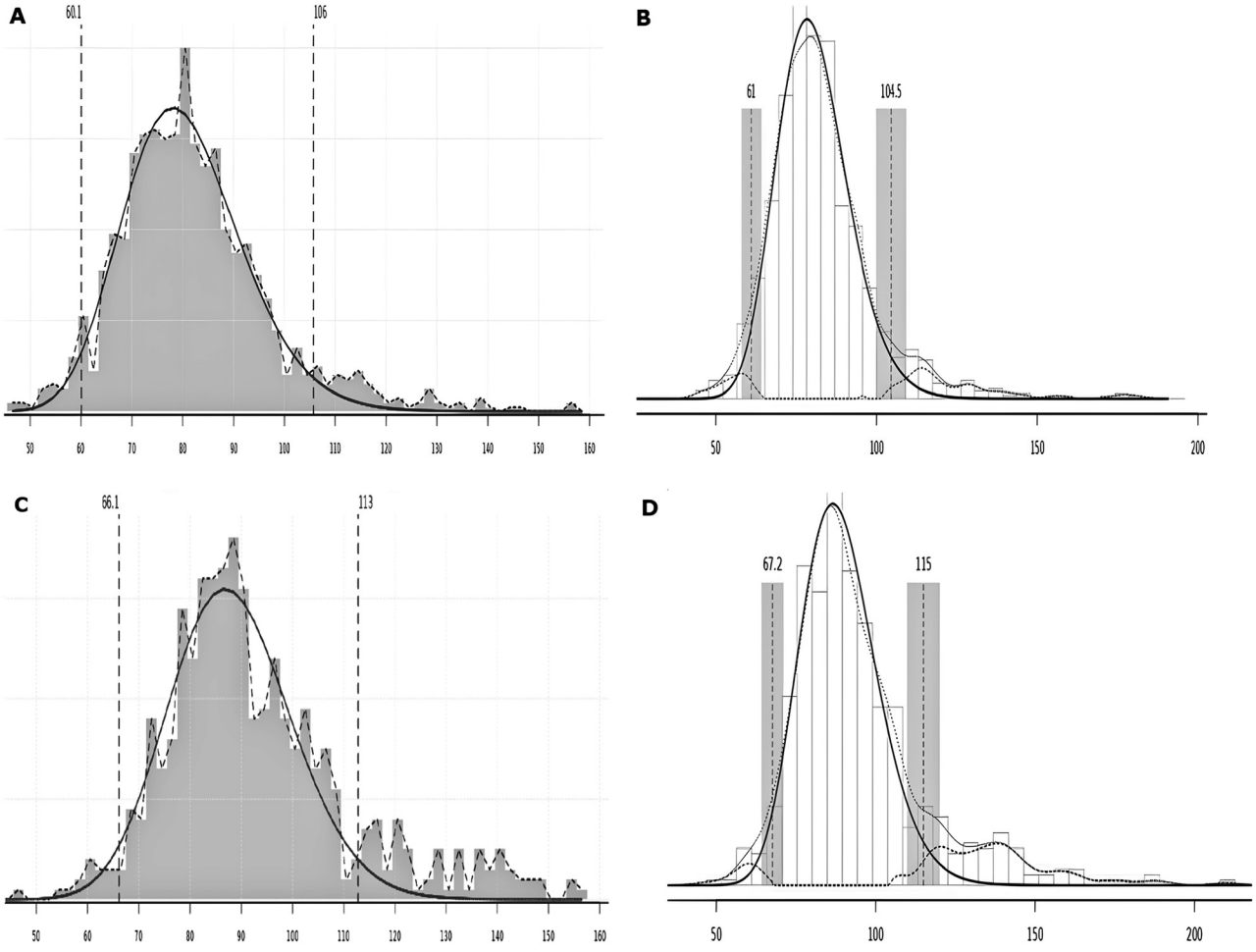

Figure 2: Graphical outcomes of the RefineR and ReflimR methods applied to both kits. (A) RefineR for Improgen kit. (B) ReflimR for Improgen kit. (C) RefineR for Archem kit. (D) ReflimR for Archem kit.

surveys also varied [17]. This may have resulted in variation in prevalences. In another study conducted in our country, the prevalence of zinc deficiency in children aged 5–16 years was found to be 27.8 %. In that study, zinc levels were measured using the ICP-MS method and the IZiNCG cut-off values were used to diagnose zinc deficiency [18]. In both studies, the prevalence of zinc deficiency was higher than the prevalence obtained using the recommended by manufacturers and determined reference intervals in our study.

IZiNCG and National Health and Nutrition Examination Survey (NHANES) data suggested cut-offs for serum zinc concentration were 65 µg/dL for <10 years age, 66 µg/dL for ≥10 years age non-pregnant females and 70 µg/dL for males [19, 20]. These cut-off values are used in many laboratories for

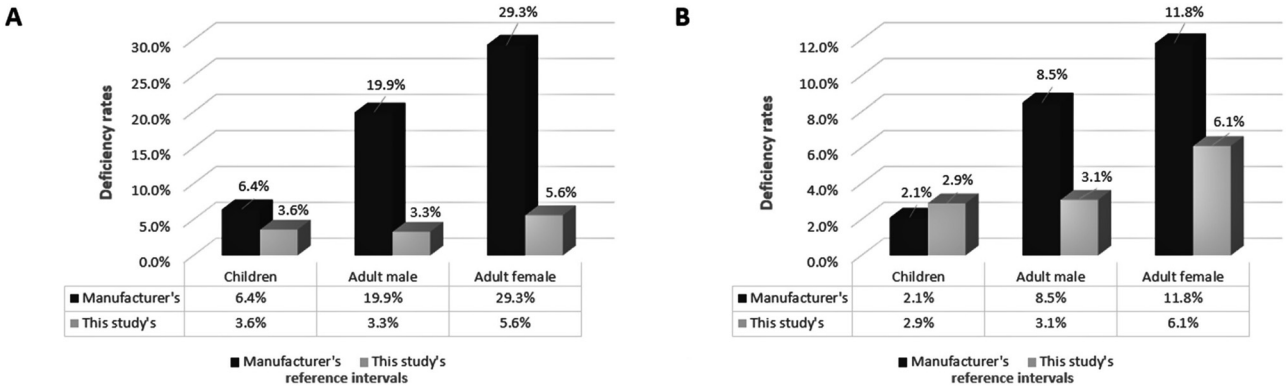

Figure 3: Prevalence of zinc deficiency according to reference intervals from two manufacturers and study-specific RefineR methods. (A) Improgen kit. (B) Archem kit.

the diagnosis of zinc deficiency. However, these values are valid for the ICP-MS method, which is the gold standard in zinc measurement. The methods employed in various zinc deficiency prevalence studies were ICP-MS and AAS. These methods have been shown to provide highly sensitive and accurate measurements of zinc levels. However, it should be noted that these methods are expensive, labour-intensive and time-consuming. Consequently, spectrophotometric methods appear to be more cost-effective and time-efficient in laboratories with a high volume of zinc tests.

In studies conducted in European countries such as Denmark, Norway, Germany, Italy and Northern Ireland; reference intervals for adults were determined as 53.6–102.6, 71.3–108.5, 68.0–149.1, 59.7–102.8 and 63.7–100.4 µg/dL, respectively [21–25]. In the studies from Italy and Northern Ireland, the 5th and 95th percentiles were accepted as reference limits [24, 25]. Among these studies, the reference intervals were calculated by indirect method only in Northern Ireland and by direct method in the others. The measurement methods employed for the determination of zinc levels were flame AAS and ICP-OES [21–25]. These studies reported reference intervals for adults without gender stratification. With the sole exception of Norway, their lower limits lay below the IZiNCG cut-offs. In Germany, however, the lower limit of 68 µg/dL exceeded the limit value for women, but remained below the value for men. It should be noted that even in studies where a similar method of measurement was used for zinc, different reference intervals were found.

The recently updated study of the NHANES data has established the zinc reference intervals as 62.1–104.6, 62.1–107.9 and 62.1–117.7 µg/dL for children, female and male, respectively [26]. These updated lower reference limits exhibited a high degree of similarity with those determined by the RefineR and ReflimR methods in our study for the Improgen® kit. In a study conducted in Türkiye using spectrophotometric method, serum Zn reference intervals were found as 66.4–104.7 in non-pregnant women, 65.6–111.5 in men and 69.2–101.1 µg/dL in pregnant women [27]. In that study, reference intervals were calculated by direct method and children under 18 years of age were not included in the study. In contrast to the previous study, the lower reference limits reported by Demirel et al. [27] were more comparable to those determined in our study for the Archem® kit using the RefineR and ReflimR methods. Collectively, these findings underscore the necessity of establishing reference intervals that are specific to both the analytical method and the population.

Although numerous statistical methods exist for calculating indirect reference intervals, our study employed only three methods. Among these, the reference intervals estimated using the Bhattacharya method were slightly lower than those obtained via the RefineR and ReflimR methods. We consider that these discrepancies may be attributed to differences variations in sample size, data distribution, and preprocessing techniques such as data cleaning. Therefore, we underscore the necessity for updated standardized guidelines to provide clear recommendations on the appropriate selection of methods based on varying data characteristics when determining indirect reference intervals.

Before clinical laboratories use the recommended reference intervals by manufacturers or guidelines, transference and local validations should be done for the validity of reference intervals. Even if reference intervals are obtained by transference, it is important to observe that they are suitable for the population and, if not, population-based reference intervals should be established by the laboratory. The reference intervals obtained by transference from different methods may be incompatible with the population due to the fact that bias was not determined by method comparison [28].

To emphasise this point, in our study we found that even the reference intervals for zinc measured

spectrophotometrically in a similar population differed. The reference intervals we determined for the Improgen® kit were lower than the reference intervals of the Archem® kit in all three methods. The overall prevalence of zinc deficiency in the similar population in different time periods was found to be 20.0 % with the Improgen® kit and 8.5 % with the Archem® kit according to the manufacturers' reference intervals. Although the determined reference intervals and lower reference limits were different, the prevalence of zinc deficiency has decreased substantially after the implementation of defined reference intervals for both kits and has reached almost the same level (20.0–4.6 % and 8.5–4.7 %). Analytical bias may explain the lower reference intervals for the Improgen® kit compared to the Archem® kit.

Zinc deficiency is rare in healthy individuals who do not have malabsorption problems and are not on a restricted dietary programme, and routine use of zinc supplements is not recommended in these individuals [29]. Furthermore, low levels of zinc in the blood do not necessarily indicate a general deficiency. Instead, they may be a reflection of the body's natural response to inflammation. Subsequent to the cessation of inflammation, blood zinc levels may return to normal [30].

The study's notable strength lies in its meticulous determination and comparison of various reference intervals for zinc levels measured spectrophotometrically with two different kits. Furthermore, the investigation extended to the impact of different reference intervals on the prevalence of zinc deficiency in a similar population. Conversely, the main limitation of this study was the inability to differentiate between pregnant and non-pregnant women, due to the fact that zinc levels decrease during pregnancy.

The establishment of appropriate and accurate zinc reference intervals is of paramount importance in order to avoid the overdiagnosis of zinc deficiency, the unnecessary laboratory testing and the administration of supplements to individuals without underlying deficiencies. Thus, unnecessary healthcare costs can be prevented.

Research ethics: Ethical approval was obtained from Prof. Dr. Cemil Tascioglu City Hospital Ethics Committee (date: 16/07/2024, approval no: 155).
Informed consent: Not applicable due to retrospective design of this study via laboratory information system.
Author contributions: Eren Vurgun: conceptualization, statistical analysis and interpretation, literature search, writing – original draft and review and editing. Ayse Aktas: data collection and processing, literature search, writing – original draft.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
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References

1. Institute of Medicine (US) Panel on Micronutrients. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academies Press (US); 2001. 12, Zinc.
2. Maywald M, Rink L. Zinc in human health and infectious diseases. Biomolecules 2022;12:1748.
3. Leoni G, Rosato A, Perozzi G, Murgia C. Zinc proteome interaction network as a model to identify nutrient-affected pathways in human pathologies. Gene Nutr 2014;9:436.
4. Maxfield L, Shukla S, Crane JS. Zinc deficiency. [Updated 2023 Jun 28]. StatPearls [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493231/[Accessed 1 March 2025].
5. Skalny AV, Aschner M, Tinkov AA. Zinc. Adv Food Nutr Res 2021;96: 251–310.
6. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr 2009;89:2040S–51S.
7. International Consortium for Harmonization of Clinical Laboratory Results. Summary of measurand harmonization activities. [Internet]. Available from: https://www.harmonization.net/measurands/ [Accessed 3 March 2025].
8. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; Approved Guideline – Third Edition. CLSI EPC28-A3c. Wayne (PA): Clinical and Laboratory Standards Institute; 2010.
9. Jones GRD, Haeckel R, Loh TP, Sikaris K, Streichert T, Katayev A, et al. IFCC Committee on Reference Intervals and Decision Limits. Indirect methods for reference interval determination – Review and recommendations. Clin Chem Lab Med 2018;57:20–9.
10. Bhattacharya C. A simple method of resolution of a distribution into Gaussian components. Biometrics 1967;23:115–35.
11. Ammer T, Schützenmeister A, Prokosch HU, Rauh M, Rank CM, Zierk J. refineR: a novel algorithm for reference interval estimation from real-world data. Sci Rep 2021;11:16023.
12. Hoffmann G, Klawitter S, Trulson I, Adler J, Holdenrieder S, Klawonn F. A novel tool for the rapid and transparent verification of reference intervals in clinical laboratories. J Clin Med 2024;13:4397.
13. ReferenceRangeR [Online]. University of Oldenburg, Germany; c2025. Available from: https://kc.uol.de/referenceranger/ [Accessed 16 June 2025].
14. Harris EK, Boyd JC. On dividing reference data into subgroups to produce separate reference intervals. Clin Chem 1990;36:265–70.
15. Haeckel R, Wosniok W, Arzideh F. Equivalence limits of reference intervals for partitioning of population data. Relevant differences of reference limits. J Lab Med 2016;40:199–205.
16. Gupta S, Brazier AKM, Lowe NM. Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. J Hum Nutr Diet 2020;33:624–43.
17. Hess SY. National risk of zinc deficiency as estimated by national surveys. Food Nutr Bull 2017;38:3–17.
18. Vuralli D, Tumer L, Hasanoglu A. Zinc deficiency in the pediatric age group is common but underevaluated. World J Pediatr 2017;13:360–6.
19. Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, et al. International zinc nutrition consultative group (IZiNCG). International

Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25:S99–203.
20. Hotz C, Peerson JM, Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). Am J Clin Nutr 2003;78:756–64.
21. Grandjean P, Nielsen GD, Jorgensen PJ, Horder M. Reference intervals for trace elements in blood: significance of risk factors. Scan. J Clin Lab Invest 1992;52:321–37.
22. Rahil-Khazen R, Bolann BJ, Ulvik RJ. Trace element reference values in serum determined by inductively coupled plasma atomic emission spectrometry. Clin Chem Lab Med 2000;38:765–72.
23. Rükgauer M, Klein J, Kruse-Jarres JD. Reference values for the trace elements copper, manganese, selenium, and zinc in the serum/plasma of children, adolescents, and adults. J Trace Elem Med Biol 1997;11: 92–8.
24. Alimonti A, Bocca B, Mannella E, Petrucci F, Zennaro F, Cotichini R, et al. Assessment of reference values for selected elements in a healthy urban population. Ann Ist Super Sanita 2005;41:181–7.
25. McMaster D, McCrum E, Patterson CC, Kerr MM, O'Reilly D, Evans AE, et al. Serum copper and zinc in random samples of the population of Northern Ireland. Am J Clin Nutr 1992;56:440–6.
26. Andrew D, Gail R, Morag B, Kishor R. Recommended reference intervals for copper and zinc in serum using the US National Health and Nutrition Examination surveys (NHANES) data. Clin Chim Acta 2023;1: 117397.
27. Demirel M, Selek S, Yildiz T, Sönmez R, Gül AZ. Determination of serum reference intervals for zinc and copper. Romanian Journal of Diabetes Nutrition and Metabolic Diseases 2023;30:42–7.
28. Ozarda Y, Higgins V, Adeli K. Verification of reference intervals in routine clinical laboratories: practical challenges and recommendations. Clin Chem Lab Med 2018;57:30–7.
29. Tsang BL, Holsted E, McDonald CM, Brown KH, Black R, Mbuya MNN, et al. Effects of foods fortified with zinc, alone or cofortified with multiple micronutrients, on health and functional outcomes: a systematic review and meta-analysis. Adv Nutr 2021;12:1821–37. Erratum in: Adv Nutr 2021;12:2040–4.
30. Wessels I, Maywald M, Rink L. Zinc as a gatekeeper of immune function. Nutrients 2017;9:1286.