

The Problems with the Establishment of Japanese Standard Reference Value of Serum Zinc Level

Yasuaki Arakawa

Japan Organization of Occupational Health and Safety, Guest
Ex-President, Japan Society for Biomedical Research on Trace Elements

Introduction

A recent analysis of Japan Society for Biomedical Research on Trace Elements indicates that the number of patients of zinc deficiency existing actually in Japan is much larger than the number that has been grasped, and that the appearance of zinc deficiency symptoms is observed at high frequency even in the state of zinc level in the serum which are exceeded the minimum value ($65 \mu\text{g/dl}$) of a preset Standard Reference Value. From these points of view, it is pointed out that there is a need for review of “standard reference values of zinc level in the serum” through the accumulation of clinical research.

There is nothing for setting of evidence-based healthy range of zinc level in the serum but to depend on expediently establishing of threshold limit value which is effective statistically and stochastically, because a nutritional decision is formed with continuous system as its basis.

Therefore, the basic concept of establishment and the current state of adoption of reference values and the problem in Japan are considered to make a plan the establishment of Japanese standard reference values of zinc level in the serum in this article.

1. The concept for the establishment of standard reference values

1) Basic principle:

In principle, a statistical processing follows the term definition 24 “biological reference interval (reference interval)” of “International Organization for Standardization (ISO) 15189 (international standard of clinical laboratory)” on quality and ability of clinical laboratory. That is to say, a statistical analysis is performed with the normal distribution 95% confidence limit (central value \pm 2SD) of inspection value (standard value) indicated by the reference individual.

2) Standard individual and standard population

Standard individual means an individual the state of health of which is selected in accordance with the properly-defined criteria and standard population means a

population which contains all of these standard individuals.

The population of reference range setting, named the reference sample group, means a set of standard individual lifestyle habits of which associated with health - such as sex, age, smoking, excessive alcohol consumption, medication, poor diet etc. - is closely examined and specimen collection of which is performed under the same conditions.

3) The establishment of a reference range:

A reference range establishment requires proper selection criteria of healthy subject, condition of blood sampling and statistical technique. The number of target samples above a certain level is needed in order to obtain statistically stable value.

4) Problem of the population

Japan Society for Biomedical Research on Trace Elements suggests that insufficient intake person of zinc may exist close to 30 percent of the whole nation in Japan. If this is the case, it will probably have a big influence not only on appropriateness of the population selection, but also on the credibility of population itself.

(Note: Reference value means the concept on the statistical processing but not clinical normal value.)

2. The problems of setting the lower limit value

1) Bases of decision in setting the lower limit value of the concentration of zinc in the serum of healthy subject.

Confirmation of evidence of decision that healthy value range of the concentration of zinc in the serum should be set to 80 to 130 $\mu\text{g}/\text{d}\ell$, that has been proposed by Japan Society for Biomedical Research on Trace Elements.

- ① There is nothing for setting of evidence-based healthy range of zinc level in the serum but to depend on expediently establishing of threshold limit value which is effective statistically and stochastically because nutritional decision is formed with continuous system as its basis.
- ② Deficiency group (range) is more widely set in order to improve the accuracy (the sensitivity)
- ③ healthy group (range) is more stringently set in order to improve the specificity
- ④ The presence of symptoms is essential for the decision of deficiency group.
- ⑤ Zinc Deficiency is diagnosed at a high frequency with in a range of 60 to 79 $\mu\text{g}/\text{d}\ell$ which is higher than 57 to 65 $\mu\text{g}/\text{d}\ell$ of healthy lower limit value that clinicians are

currently using as criterion for judgment.

⑥ This establishment is matters calling for special attention in the clinical diagnosis, It is recommended to be noted as special mention in the assessment criterion of the inspection data.

2) Proposal of Japan Society for Biomedical Research on Trace Elements¹⁻¹⁰⁾

① In the clinical examination at the preventive stage without a medical practice, it should be noted with the proviso that healthy value range with higher accuracy and specificity is 80 to 130 $\mu\text{g}/\text{d}\ell$ (the healthy lower limit value is 80 $\mu\text{g}/\text{d}\ell$) and it is essential to confirm “the presence of zinc deficiency symptoms” in case of the range of 60 to 79 $\mu\text{g}/\text{d}\ell$. Alternatively, the range of 60 to 79 $\mu\text{g}/\text{d}\ell$ should be displayed with characters or the like for calling the attention of a subject as the boundary region (subclinical zinc deficiency or zinc deficiency state).

② In case of the treatment and diagnosis, the healthy lower limit value is more stringently set to 80 $\mu\text{g}/\text{d}\ell$ in order to improve the accuracy and the specificity of diagnostic criteria for deficiency.

3) It is a comment of Japanese Society of Laboratory Medicine that in the current situation that clinical symptom of zinc deficiency is not clear, an establishment of the lower limit value is not appropriate.

4) Problem of zinc intake

Japan Society for Biomedical Research on Trace Elements suggests that insufficient intake person of zinc may exist close to 30 percent of the whole nation in Japan. (This problem has a big influence not only on the population selection, but also on the credibility of population itself.)

5) To the proposal of Japan Society for Biomedical Research on Trace Elements, Japan Registered Clinical Laboratories Association responded that in the current situation, the Association adjusts itself to opinion of Japanese Society of Laboratory Medicine. The Associate Member Center has set the healthy lower limit value as 60 to 70 $\mu\text{g}/\text{d}\ell$ based on its own statistical processing.

3. The difference in reference values is due to the measurement method

1) The need for standardization of measurement method:

It has been pointed out that there are differences in the measurement method and the measured value among the inspection facilities, that is to say, the discrepancy in clinical test data that occurred among the respective facilities. Quality control in the clinical inspection facilities contains an internal quality control and an external quality control.

Not only an internal quality control in the individual inspection facilities but also an external quality control targeted the respective facilities need to be conducted over a wide area, under the common conditions, together with affiliate of medical inspection such as Japan Medical Association, Japanese Association of Medical Technologists, Japan Registered Clinical Laboratories Association, Japanese Society of Clinical Chemistry, Japanese Society of Laboratory Medicine, International Federation of Clinical Chemistry and Laboratory Medicine, IFCC, and that an approach to the standardization need to be embodied by mutual cooperation with affiliate.

2) Selection of standard methods

The examinations such as the following are required for the selection of the standard method.

- ① The distribution of mask specimen and the comparison of measurement value according to various measurement methods and the selection of standard methods
- ② Quality control in the standard method adopted facility

3) International comparison

Both domestic and foreign, it is necessary to compare the measurement method and to select a reliable method in each country, in order to obtain an accurate measured value or the sharable reference value.

4) Sharing of the reference range

It is most desirable for the reference range to be shared through standardization of the measurement method.

4. Current state of the reference value adopted in Japan

As shown in Table 1, a questionnaire survey of the situation of adoption of the reference value was conducted on councilors of Japanese Society of Laboratory Medicine and Associate Member Center of Japan Registered Clinical Laboratories Association.

1) Adoption of the reference value:

According to the survey, the adoption of the reference value is largely divided into two cases: where it depends on literature values and where it depends on original reference value of each manufacturer. The range of the reference value which has been adopted in a variety of facilities is scattered in the range of 59 to140 $\mu\text{g}/\text{d}\ell$ such as 59 to135, 65 to110, 70 to110, 66 to118, 80 to130 and 80 to140 $\mu\text{g}/\text{d}\ell$.

2) The setting of the reference values based on its own grounds in the

measurements institutions and the facilities:

It is difficult to change the reference value that has been set on the basis of its own grounds in the individual inspection facility.

5. Selection of the target for measurement

1) Selection of healthy group

- ① It is important to clarify a definition of healthy person and to select the healthy standard population with the same conditions in the sex, age, lifestyle habits, specimen collection, etc.
- ② It is important to properly determine whether or not the understanding of the healthy state or disease state is appropriate and whether or not the clinical diagnostic criteria are clear. (Normal value and healthy value differ in the definition: The definition and criterion of “normal” is ambiguous. A healthy person is not always a guarantee of a normal person.)

2) Selection of deficiency group:

The presence of symptoms is essential for the decision of deficiency group. Therefore, even if a measured value is more than the setting healthy lower limit value, a subject is diagnosed with healthy serum zinc deficiency (or serum zinc positive-zinc deficiency), if the deficiency symptoms are recognized.

6. Clinical significance of serum zinc

Upon the setting the reference value, it is most important to consider and make a clear about the clinical significance of serum zinc, such as the following.

- 1) To consider and clarify what extent the serum zinc concentration reflects disease state and manifestation.
- 2) To consider and clarify what extent the serum zinc concentration reflects tissue zinc and zinc state at the point of action.

7. Clinical significance of tissue zinc and development of measurement method

In considering the clinical significance of serum zinc, such as the following, it is important to grasp the clinical significance of the tissue zinc in parallel with the serum zinc. Thereby, it must be also investigated for not only zinc state in the tissue and at the point of action but also relevance of serum zinc concentration and tissue zinc concentration. Moreover, in order to fulfill that, a development of measurement method for zinc in the tissue is necessary.

- 1) Grasp of zinc state in the tissue and at the point of action
- 2) Confirmation of the presence of local zinc deficiency
- 3) Development of measurement method for zinc in the tissue

8. International comparison

Also in the international as well as domestic, it is necessary to compare the measurement method and to select a reliable method in each country, in order to obtain an accurate measured value or the sharable reference value. Especially, to aim for the international standardization, it is necessary to make a comparison of the measurement method and the reference value set in accordance with the criteria of ISO and IFCC, or a guideline of CCLS, together with the different Dietary Reference Intakes of Zinc in each country, such as the following.

- 1) Measurement method
- 2) The reference value
- 3) Dietary reference intakes of zinc
- 4) International standardization set in accordance with the criteria of ISO and IFCC

9. Guidance in the use of a reference range

- 1) Proper use of clinical judgment value in the use of a reference range

It is necessary to use properly such clinical judgment value as pathology identification value (cutoff value), therapeutic target value and prophylactic value for different purposes in accordance to the use.

- 2) Sharing of the inspection information of the patient

It is most desirable for the inspection information of the patient to be shared among the medical institution (inspection institute)

- 3) Common use of the reference range

It is most desirable for the reference range to be commoditized through standardization of the measurement method.

10. Problems in the case of changing the reference value

- 1) In the case of changing the reference value through Japan Registered Clinical Laboratories etc., it is necessary to indicate clearly the reason for needing change towards all customer medical institution throughout the country, and that is also accountability to them as to the reasons for the change. If this process will be not performed reliably, the difference with the past data may gush out and may cause

the confusion of clinical practice.

- 2) It is necessary to clearly indicate the convincing evidences such as reasons for the change, contents of the change, numerical value which become the basis of the setting (publication of scientific papers etc.)

Note: Criteria for selection of blood donor in the reference range setting

Upon the selection of blood donor for reference range setting, in order to reliably identify an individual of healthy volunteers, it is required to fill out the medical questionnaire, to confirm by interviewing with a subject, to clarify lifestyle habits, past medical history, vitamins taking, and to exclude curative medicine recipient, pregnant woman, CBC abnormal person, urine qualitative inspection abnormal person, smokers of more than 20 cigarettes a day, person having the BMI value more than 25 (criteria of obesity), habitual drunkard of more than 2 goes (180 ml/go) (refer to units of Japanese sake) a day in terms of sake, and further in a more stringent selection, drinker (γ -GTP), person within postprandial 9 hours (TG), person within postprandial 3 hours (GLU), person doing vigorous exercise from the sample.

Blood collection of the specimen contingent on fasting in the early morning and the measurement is conducted under condition of the same facilities and the same condition. Table 2 shows the criteria for selection of blood donor in the reference range setting.

Table 1 Current state of the reference value adopted in Japan

	Adoption of the reference value	Number
About measurement	① measured in its own facility	18
	② outsourcing	24
About reference value	③ Using the reference value of its own facility setting	7
	④ Using the reference value of manufacturer	34

* A questionnaire survey of the adoption situation of the reference value was conducted on councilors of Japanese Society of Laboratory Medicine and Associate Member Center of Japan Registered Clinical Laboratories Association.

[Comments from each facility] (extracted and paraphrased)

- ▶ Using the reference value (59 to135 $\mu\text{g}/\text{d}\ell$) set by the BML.
- ▶ Previously measured in its own facility using atomic absorption spectrometry. However, it does not meet profitability for expensive equipment and low inspection frequency.
- ▶ Using atomic absorption spectrometry, the reference value is 59 to135 $\mu\text{g}/\text{d}\ell$ (setting basis is the in-house SRL set).
- ▶ Using the reference value (65 to110 $\mu\text{g}/\text{d}\ell$) set by the subcontractor.
- ▶ The reference value range is 65 to110 $\mu\text{g}/\text{d}\ell$.
- ▶ Outsourcing to SRL, using the reference value (65 to110 $\mu\text{g}/\text{d}\ell$) set by the SRL.
- ▶ Using colorimetric method with zinc kit, the reference value is 65 to110 $\mu\text{g}/\text{d}\ell$ (setting basis is the in-house Shino-Test Corporation set).
- ▶ Quotation from the literature values (66 to118 $\mu\text{g}/\text{d}\ell$).
- ▶ Using the reference value (70 to110 $\mu\text{g}/\text{d}\ell$) of the in-house setting.
- ▶ Quotation from the literature values (80 to130 $\mu\text{g}/\text{d}\ell$).
- ▶ Using the reference value (80 to130 $\mu\text{g}/\text{d}\ell$) set by the manufacturer.
- ▶ Measured in its own facility using a hand method, Using the reference value (80 to140 $\mu\text{g}/\text{d}\ell$) of the in-house setting with reference to the literature values and opinion of the clinical practice.
- ▶ Using the reference value (80 to140 $\mu\text{g}/\text{d}\ell$) of its own facility setting.
- ▶ Adoption of the reference value quoted from the literature.
- ▶ Using the reference value set by the manufacture, which has set a different reference value for each time zone. Use the morning time zone.
- ▶ Using the reference value set by the subcontractor.
- ▶ Plan to start measurement of in-hospital from April, Intend to adopt the reference value of outsourcing company to avoid the confusion of clinical practice.
- ▶ Outsourcing to SRL, Using the reference value (65 to110 $\mu\text{g}/\text{d}\ell$) set by the SRL
- ▶ Until setting up the reference value in its own facility is completed, using the reference value (65 to110 $\mu\text{g}/\text{d}\ell$) set by the SRL which is the inspection contractors.
- ▶ Outsourcing to BML, Using the reference value (59 to135 $\mu\text{g}/\text{d}\ell$) set by the BML.
- ▶ Outsourcing to MBC, Using the reference value set by the MBC.

*** Abbreviation:**

BML: Bio Medical Laboratories, Inc.

SRL: Superconductivity Res. Lab Special Reference Laboratories, Inc

MBC: name change; LSI Medience Corporation

Additional information

We are newly formulating the Standard Reference Values of Zinc Level in the Serum using mass health examination. This detail will be published as original paper near future.

In summary, the findings is that using colorimetric method by zinc measurement kit "ACCURAS AUTO Zn" (the reagent is applicable to all auto-analyzers) with accuracy equivalent to or better than that of atomic absorption spectrometry, the well-trusted reference range and reference value are established by the reference value development committee (working as a chair) of Japanese Society for Zinc Nutritional Therapy involved as an advisor.

At the present time, to summarize the results from the 3 facilities, the reference value is set to $83.7 \pm 22.4 \mu\text{g/dl}$ and the reference range is set to $61.3 \sim 106.1 \mu\text{g/dl}$ as common reference in the surveys of the population consisting of 911 subjects (men: 476 subjects, women: 435 subjects), and its average age: 51.5 years old (20~92 years old).

Table 2 Criteria for selection of blood donor in the reference range setting

Upon the selection, it is necessary to be a person who meets the following criteria.

- 1) A person who has not a past medical history and a disease currently in treatment, Except for a person such as having a medical examination or being under medical treatment for some disease and a person with chronic disease such as diabetes, inflammatory diseases, liver disease, gout and M protein appearance, Except for a person who takes medicines, Except for a person who has the following abnormal data such as M protein, $\text{WBC} > 8000/\mu\text{l}$, $\text{CRP} \geq 0.3 \text{ mg/dl}$, $\text{ChE} < 0.6 \angle \text{pH}$, $\text{TP} \leq 6.6 \text{ g/dl}$, $\text{Alb} \leq 3.8 \text{ g/dl}$, $\text{LDH} > 470 \text{ IU}$, $\text{AST} \geq 32 \text{ IU}$, $\text{ALT} \geq 29 \text{ IU}$.
 - 2) A normotensive subject (a systolic maximum pressure $\leq 159 \text{ mmHg}$, a diastolic maximum pressure $\leq 94 \text{ mmHg}$).
 - 3) A person that degree of obesity is normal, in other words, is not extremely skinny and obesity. Ideal weight is set to $\text{BMI} = 22$, an acceptable range is that within $\pm 10\%$. (defined respectively, obesity $\geq \text{BMI} 25$ and low weight $< \text{BMI} 18.5$). Except for a person that BMI is 25 or more.
 - 4) A person of less than 2 goes (180 ml/go) (refer to units of Japanese sake) a day in terms of sake, (or a small bottle of beer and less than 1 go a day in terms of sake) , or of no drinking state.
 - 5) A smoker of less than 20 cigarettes a day, or a person who do not have a smoking
-

habit.

- 6) A person who is negative in the test for glucose and protein in the urine, and who is glucose level of 110 mg/dℓ or less and urea nitrogen (BUN) of 22 mg/dℓ or less in the blood.
- 7) A person with normal Hb values (man:13.0 g/dℓ or more, woman:11.0 g/dℓ or more) and without anemia.
- 8) A person who is normal in the liver function test for AST, ALT, and γ -GTP).

*The selection of blood donor shall meet the criteria equivalent to the guidelines of CLSI (Clinical and Laboratory Standards Institute, Former; NCCLS (National Committee for Clinical Laboratory Standards) and the consent of the person shall be obtained.

References

- 1) Tomita H: A proposal of the clinical standard value for diagnose of zinc deficiency by serum zinc value (in Japanese). *Biomed Res Trace Elements* 19(1): 22-24, 2008.
- 2) Tomita H, Tanaka M, Ikui A: Clinical standard value for diagnosis of zinc deficiency by the serum zinc value on the basis of evidence (in Japanese). *Biomed Res Trace Elements* 18(1): 54-62, 2007.
- 3) Kurasawa R, Kubori S: Zinc Deficiency and its clinical features in the cases found in Kitamimaki, a rural area in Japan (in Japanese). *Biomed Res Trace Elements* 17(2):91-93, 2006.
- 4) Kurasawa R, Kubori S, et al: The symptoms and pathogenesis of zinc deficiency. *Biomed Res Trace Elements* 21(1):1-12, 2010.
- 5) Kurasawa R, Kubori S, et al: Diagnosing zinc deficiency with greater efficacy by upwardly adjusting the serum zinc level limit to 80 μ g/dℓ (in Japanese). *Biomed Res Trace Elements* 22(1):34-37, 2011.
- 6) Yokoi K, Egger NG, et al: Association between plasma zinc concentration and zinc kinetic parameters in premenopausal women. *Am J Physiol Endocrinol Metab* 285: E1010-E1020, 2003.
- 7) Yokoi K: Human zinc kinetics using stable isotope tracers, with a special emphasis on the estimation of the critical value for plasma zinc in humans (in Japanese). *Biomed Res Trace Elements* 20(4): 284-295, 2009.
- 8) Arakawa Y as the president of the Society: "About setting the healthy lower limit value of serum zinc level" –Proposal from the Japan Society for Biomedical Research on Trace Elements (in Japanese) – , Correspondence with Japan Registered Clinical Laboratories Association and workshop report. *Biomed Res Trace Elements* 21(1): 43-48, 2010.

- 9) Arakawa Y: About setting of “the healthy lower limit value of serum zinc level” by the Japan Society for Biomedical Research on Trace Elements (in Japanese). Biomed Res Trace Elements 21(3): 182, 2010.
- 10) Arakawa Y: The matters pertaining to the establishment of Japanese standard reference values of zinc level in the serum (in Japanese). Biomed Res Trace Elements 23(3): 217-220, 2012.

(Though a subject regarding this matter was already described in Japanese as “Letter to editor” in Biomed Res Trace Elements 23(3): 217-220, 2012, the present article is one that has been described in more details about the subject matter of similar kind in English.)