Review article

The Pre Analytical Phase: Precautions in Specimen Collection and Patient Preparation for Trace and Ultra Trace Elements Analysis

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Abstract

Pre analytical factors are probably the most important causes of erroneous trace element reference data in biological matrices today and the development of sensitive , specific, and accurate analytical technology at an acceptable cost has moved determination of trace and ultra trace elements from research facilities into a wide range of clinical laboratories. Besides, expanding knowledge of trace element nutrition and toxicity has increased clinical demand for these assays. However, with increased sensitivity and lower limits of detection the problem of specimen contamination with the element of interest has been magnified. It is vital that the accurately determined trace element concentration to reflect the condition of the patient and not contamination introduced during collection and handling. In the following paper, the concept of pre analytical factors that can contribute to trace and ultra trace elements concentration are discussed. In addition, specific percussions for collecting different sample types and special considerations in patient preparations and sample types for specific Trace and Ultra Trace Elements analysis are addressed.

Keywords: *: Trace Elements- Pre analytical Phase-Anticoagulated- Specimen collection*

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Introduction

Trace Elements are defined as elements that occur at a level of 0.01 to 100 µg/g (10 µg/L to 10^4 µg/ L) (1). Arbitrary elements at a level of less than 0.01 µg/g (less than 10 µg/L) are called Ultra Trace Elements. The concept that a specific trace element is consistently detectable in human tissues or fluids does not imply that it is essential. Many trace elements are so ubiquitous in the environment (e.g. Al, Pb) and surprisingly they are normally found in human tissues and fluids. An element is considered essential if without it the species cannot achieve normal, healthy growth or complete its normal life cycle. Furthermore, if it is part of a molecule of an essential constituent or metabolite that exerts its effect directly on growth or metabolism and not by some indirect effects (2).

Sample contamination errors are common in trace elements analysis and can occur at any stage of the pre analytical process, including proper training of an individual prior to specimen collection, sample collection, transport, handling, and sample preparation (drying, homogenization, diluting and etc). Therefore, special precautions should be followed to eliminate or at least reduce and control the errors . In addition, depending on the specific element being analyzed, its natural abundance , and the concentration level in the sample precautions may differ (1-2). In the present paper first, the pre analytical principle factors for trace and ultra trace elements analysis will be discussed. Then, specific percussions for collecting different sample types and last, special considerations in patient preparations and sample types for specific Trace and Ultra Trace Elements analysis are addressed.

1. Pre Analytical Principle Factors for Trace & Ultra trace Elements Analysis

Generally, if analysis for ultra trace & trace elements are to have any validity, a detailed attention should be paid to the collection devices , anticoagulant selections, and collection protocols. It is important that the laboratory does not allow specimens for trace element determinations to be sampled for other tests outside the trace element laboratory section until the trace element determination has been performed or an adequate sample has been processed for this purpose. Furthermore, before collection, handling and storage, devices that have been validated for the

element of interest by the laboratory performing the analysis should be used and each new lot of materials including chemical powders, reagents, anticoagulants and other consumables used in the collection should be checked so that they will not contribute to the contamination of the specimen. This check can eliminate the random contamination errors. It should be ensured that systematic contamination also has not occurred and personnel responsible for collection are properly trained. In addition, only non powdered gloves throughout the collection procedure should be used and blood samples in glass tubes never should be kept frozen because the glass can fracture and cause the tube contents to leak out (3-5). All laboratory glassware and plasticwares should be acid washed by soaking overnight in solution of 10% (v/v) nitric acid and then given a final wash with type I water (6,7)

Use of high grade de-ionized water is essential for quality trace element analysis. Various parameters are used to characterize the quality of de-ionized water. However, for trace elements analysis the resistivity of the water is a reasonable guide to its quality. Typically, a resistivity of greater than 10 megohme per centimeter (MΩ) at 25 ^{0}C is considered type I water by several organizations (6,7). In case of anticoagulant selection, Ethylenediaminetetraacetate (EDTA) is the preferred one when specimens are to be transported with more than 36 hours delay. However, as a good chelating ligand, EDTA is easily contaminated with metal ions during the manufacturing process. Heparin is less likely to be contaminated and can be obtained as "trace element free". But heparin is generally considered a less reliable anticoagulant for long duration (24 to 36 hours). Often the choice between EDTA and heparin is based on known problems with the analytical methods. Furthermore, in ultra trace elements determinations, samples drawn through a Teflon tube, the initial drew should not be used. The detailed information on skin puncture and sample collection procedures can be found in NCCLS documents (8,9,10).

2. Specific Percussions for Collecting Different(specimen) Sample Types

Any specimen collection protocol depends on the target trace or ultra trace element being determined and careful consideration should be given to the kind of samples required for the analysis including whole blood, serum, or plasma. For lead, cadmium, and mercury whole blood is the specimen of choice. For most others, serum or plasma is preferred. Furthermore, for the diagnostic tests venous blood is the preferred sample in determination of some elements because it is unlikely to be compromised by contamination. Blood especially from young children is more convenient to obtain using the fingerstick method ,however, this method is more prone to contamination errors and cleaning of the site is required.

2.1.Whole blood, plasma, serum & urine

Only evacuated anticoagulated tubes that are specifically designed for trace elements collection and are checked by the laboratory are suitable tubes for trace analysis. If a series of tubes are to be collected from the same site, to minimize contamination, use the initial specimen for other tests and collect the blood for trace elements last. After blood collection the tubes should be inverted several times to thoroughly mix the anticoagulant with the blood (11,12) and only stainless steel needles with no aluminum or other metal should be used. To separate the plasma from the erythrocytes and leukocytes, Centrifuge tubes for 10 minutes at a relative centrifugal force (RCF) of 1,000- 1,200 x g. Remove the stopper and pour plasma into an acid-washed, plastic, screw-topped tube. Do not touch plasma with any utensils, unless they have been acid- washed .If transfer pipets are used , they should also be acid washed.

Standard red- topped evacuated tubes or plastic syringes with black rubber tipped plungers are grossly contaminated with zinc and other trace elements and are not acceptable for the serum collection in determination of trace elements. Suitability of a given product should always be evaluated for the specific element to be tested. After collection, allow the blood to clot for at least 30 minutes at room temperature. Then, centrifuge the tubes for ten minutes at an RCF of 1,000 -1,200 x g to separate the serum from the clot. Remove the stopper and pour the serum into an acid-washed , plastic, screw- topped tube.

Urine excretion may be used for the monitoring of heavy metal overload in following up some therapeutic mobilization tests such as use of Desferal (Fe and Al), EDTA (Pb), or D penicilamine (Cu) and for the investigation of a deficiency mechanism. The laboratory may ask for any of the following urine samples (13).

2.2. 24 hour urine collection

If urinary excretion data for trace element analysis is required, 24 hour urine collection is the preferred specimen. But results of 24 hour urine should be corrected for creatinine. Urine collection should be arranged away from the suspected exposure site. If workplace exposure is suspected, the collection should be taken during off hours, preferably days without any occupational exposure and away from the work environment. The patient should be provided with an acid- washed, wide-mouthed voiding plastic collection container. Metal or

porcelain collection containers should not be used. It is also important to not to use the plastic jars with metal lids typically found in hospital settings or urinalysis laboratories. Furthermore, the collection container should be kept refrigerated during the 24 hour collection period.

For some trace element determinations (13), urine needs to be stabilized by addition of ultrapure nitric acid to the collection container to lower the pH to less than (<2). Usually 20 mL of 6 mol/L nitric acid is adequate for the stabilization. The urine sample should be mixed thoroughly and then a 50-100 mL aliquot in a trace element free cylinder should be measured and transferred into acid washed plastic, screw capped bottles. To obtain 24 hours volume. measure the remaining volume before discarding it .

2.3. Random (Spot) Urine

Random urine collection is sometimes used for screening (arsenic, mercury) in cases of occupational exposure. The simultaneous measurement of urinary creatinine may assist with the validity of the results. A random urine void may be collected by using a suitable plastic container with a plastic lid. However, before using, their suitability for the element of concern should be validated. The entire urine spot collection can be delivered to the laboratory or a small portion (5 mL) can be poured off into a plastic screw cap vial and sent to the laboratory. In this case it is not necessary to acidify the sample and it can be frozen.

2.4. Tissues

In particular, for trace element quantization of soft tissues, the liver is used in the diagnosis of disorders such as hemochromatosis (iron) and Wilson's disease(copper). For hard tissues bone may be used in the assessment of aluminum or lead poisoning. For all types of tissue the preferred storage container is a plastic, acid-washed, screw capped vial or a 50 mL screw capped, plastic centrifuge tube. In addition, selection of appropriate tissues and their quantities vary based on the element of interest within the tissue and also analysis. Therefore, the laboratory should use a specific protocol for the collection of tissues in each element analysis.

3. Patient Preparations and Sample Types for Specific Trace and Ultra Trace Elements Analysis.

Depending on any specific trace or ultra trace element being determined careful consideration should be given to another pre analytical factor such as patient preparation.

3.1- Aluminum

Aluminum accumulation/toxicity is monitored in patients with renal failure, particularly those on hemodialysis treatment.

a. Specimens

Serum ,24 –hour urine , bone special fluids (dialysate fluid and water).Urine aluminum is used for occupational exposure and its concentration correlates with current air levels and number of exposure years. Because this type of aluminum exposure has not been shown to be hazardous, the value of urinary monitoring is debatable. However, bulk analysis of ashed bone, or histochemical localization of aluminum at the mineralization front, provide an excellent means of assessing the body burden of aluminum to assess aluminum toxicity in patients with chronic renal failure (14). Collection of specimens for serum aluminum analysis can be a complicating factor. Most of the common evacuated blood collection devices used in phlebotomy today have rubber stoppers that are made of aluminum silicate and simple puncture of the rubber stopper for blood collection is sufficient to contaminate the sample with aluminum. Therefore, special evacuated blood collection tubes are required for aluminum determination (15).

b. Patient preparation

Limiting oral ingestion of fruit juices and tea 24 hours before blood collection is recommended because oral citrate enhances GI aluminum absorption which result in increased blood concentrations (16)

3.2- Arsenic

The major cause of concern with arsenic is the potential acute and chronic toxicity of its compounds to human.

a. Specimen

If significant exposure or intentional exposure is suspected, a random urine specimen collected in an office and submitted to the laboratory can document the presence or absence of arsenic. Urine collection should be arranged away from the suspected exposure site. If work place exposure is suspected, the collection should be taken during off hours away from the work environment, preferably on the weekend. Arsenic determination in serum, plasma, or whole blood, are of little value because the half lives of arsenic species in blood appear to be short (17,18) .

b. Patient preparation

The patient should not consume seafood for several days before the collection.

3.3- Cadmium

The health risks arising from cadmium are greatest when inhalation of cadmium from occupational sources results directly in lung damage. In addition, between 10 to 50% of inhaled cadmium is absorbed, while only 5% of ingested cadmium is absorbed from the gastrointestinal (GI) tract. Therefore, the major concern with cadmium is

assessing industrial exposures. In addition, smokers have twice the blood cadmium concentration seen in nonsmokers. Generally in long term, low level exposure and a urinary cadmium concentration above 10 µg/L or 10 mg/kg creatinine signals impending or actual tubular impairment. A blood value above 10 µg/L implies that cadmium exposure of a significant degree has taken place and the half life of cadmium in the body is 10 to 30 years (19,20).

a. Specimen

Random urine, and whole blood are specimens of choice. Most of the cadmium in blood is in the erythrocytes and presence of increased amounts of low molecular weight proteins(e.g. β micro-globulins) in urine can be used as an early indicator of exposure. Because cadmium can be present in steel and in added anticoagulants, the cadmium content should be checked before choosing a particular collection device or container.

b .Patient preparation

Urine samples should be collected outside the industrial environment and an early morning urine sample is favored for sufficient concentration of cadmium.

3.4- Chromium

Another ultra trace element that linked with industrial exposure is chromium and concerns with identifying workers who fail to adhere to recommended working practices (21).

a-Specimen

Blood and serum chromium concentrations are increased in workers exposed to it. However, 2 hour urine collection is the preferred biological specimen for biological monitoring purposes (22).

b. Patients preparation

There are no specific recommendations. However, for occupational exposure, specimen collection should be done away from the workplace, preferably over the weekend.

3.5-Cobalt

Workers who fail to adhere to recommended working practices will suffer from chronic cobalt toxicity. Chronic cobalt poisoning can manifest as cardiomyopathy, lung, skin , GI tract symptoms, hematological disorders, and thyroid disease (23).

a. Specimen

Serum is the preferred biological specimen.

b.Patient preparation

Consumption of beer should be avoided.

3.6- Copper

Copper (Cu) is an essential trace metal found in all living organisms in the oxidized Cu(II) and reduced Cu(I) states. It serves as an important catalytic cofactor in redox chemistry for proteins that carry out fundamental biological functions for growth and development. Determination of copper helps in the diagnosis of acquired copper deficiency including malabsorption, malnutrition, genetic copper deficiencies, and acquired toxicity such as occupational exposure and accidental copper toxicity (24-25).

a-Specimen

Serum is used to assess a person's copper status. However, there is a diurnal variation with the highest copper levels observed in the morning. Hypercupremia occurs in liver disease, infection and inflammation, trauma, or certain neoplasms, oral contraceptives elevates copper levels by increasing ceruloplasmin concentration. A 24 hour urine is used in diagnosing or assessing treatment for Wilson disease (copper overload). The level of copper found in the urine specimen after a penicillamine is used in the diagnosis of Wilson disease(25) . In addition, copper found in liver tissue also may be quantitated in the assessment of Wilson's disease.

b.Patient preparation

To eliminate the effects of diurnal variation, collect samples at the same time each day.

3.7- Iron

Assessment of iron deficiency and overload might best be made by a combination of iron, transferring saturation, and ferritin. Transferrin saturation of >80% is expected in hemochromatosis (24,26).

a.Specimen

Serum is the preferred sample for assessing deficiency or toxicity of iron. 24 hour urine sample is to monitor chelation therapy, and liver biopsy is for diagnosis of hemochromatosis.

b.Patients preparation

To eliminate the effects of diurnal variation, fasting morning serum samples at the same time each day should be collected.

3.8- Lead

The major concern with lead is its potential acute and chronic toxicity, diagnosis of occupational or environmental exposure and also screening for excess lead exposure in children(27, 28).

a.Specimen

Generally it is accepted that whole blood is the most reliable index of recent exposure to lead. For diagnostic purposes, venous blood should be analyzed ,but for pediatric screening purposes, a capillary liquid blood lead level obtained using the fingerstick method is acceptable. In addition, for lead mobilization test, special collection instruments recommended for collecting an 8 hour

urine sample. This 8 hour urine sample is collected immediately after administering to the patient a dose of CaNa2EDTA.

b.Patient preparation
The only preparation required is to ensure that the site of collection is properly cleaned before puncture. This is especially important when collecting blood for screening purposes using the fingerstick method, because contamination errors can be large

3.9- Manganese
Manganese is both an activator and a constituent of several enzymes. Furthermore, is another element that is concerned with industrial exposure (29, 30).
a.Specimen

The sample of choice for analysis is serum. Packed blood cell levels of manganese are approximately 25 times higher than plasma/serum levels. Therefore, partially hemolyzed blood samples will yield plasma samples contaminated with intracellular manganese.

b.Patient preparation
The specimen collection should be obtained away from the workplace, preferably over the weekend.

3.10- Mercury
The toxicological features of mercury reflects its three forms: elemental, inorganic and organic compounds(31-33). Inorganic compounds may contain mercury in oxidation states (+1) or (+2). Inorganic mercury compounds or salts are weakly absorbed by the GI tract and they are rapidly eliminated from the blood to the kidney and liver. The organic compounds circulate in the blood for a long time and they gradually accumulate in the central nervous system. Furthermore, methyl-mercury type compounds are rapidly absorbed in the GI tract and they accumulate in the red blood cells.

a. Specimen
In the assessment of exposure to inorganic mercury compounds or salts, urine is the most suitable specimen for analysis. To assess organic mercury compounds, whole blood is the most suitable specimen for analysis. For methyl-and ethyl-mercury type compounds, the blood half life is significantly longer than for inorganic compounds.

b.Patient preparation
None is required.

3.11- Molybdenum

The three principal molybdenum containing enzymes of human and animal tissues, namely Xanthine dehydrogenase/oxidase, Aldehyde oxidase and Sulfate oxidase share a common cofactor " molybdopterin". A nutritional deficiency of molybdenum gives rise to clinical symptoms suggestive of a deficiency of sulfate oxidase (34). Furthermore, it is also involved with the assessment of occupational exposure.

a.Specimen
Serum is the sample of choice.

b-Patient preparation
Obtain the specimen away from the workplace, preferably over the weekend.

3.12- Nickel
Workers who fail to adhere to recommended working practices will suffer from occupational exposures (35) .

a.Specimen
24 hour urine, and serum sample are the analysis samples. Nickel concentrations in urine or serum specimens taken from workers with inhalation exposures to soluble nickel salts reflects primarily the amount of nickel absorbed during 1 to 2 preceding days. For less soluble nickel compounds, nickel concentrations in serum or urine reflect the combined influences of recent exposures.

b.Patient preparation
Obtain the specimen away from the workplace, preferably over the weekend.

3.13- Selenium
Determination of selenium is concerned with its assessment in deficiency or toxicity cases. Only low selenium concentrations in serum correlate well with glutathione peroxidase status. It appears that whole blood selenium analysis is of little value. Reference ranges for selenium are age specific and they may vary by geographical location (24,36).

a.Specimen
For determining selenium in urine , a 24 hour urine collection is recommended.

b.Patient preparation
None is required.

3.14- Zinc

After iron, zinc is the most abundant trace element in the body and as such, the normal concentrations

of zinc in biological samples is relatively high. Zinc is an essential component of many important enzymes including alcohol dehydrogenase, caronic anhydrase, alkaline phosphatase, procarboxy peptidase, and superoxide dismutase. The most common forms of zinc deficiency are from malnutrition, excessive zinc excretion, or malabsorption. Stress and tissue destruction from severe burns, surgery or trauma can also cause zinc deficiency. Zinc toxicity may result from industrial exposure to dust or metal fumes. Consumption of acidic food and beverages packed in galvanized cans has also been reported as a source of excess zinc exposure (24,37-38).

a.Specimen
In the case of suspected zinc deficiency (inherited or acquired) or toxicity serum and urine are used for determination of zinc level.
b.Patient preparation
To avoid the effects of diurnal variation, a morning fasting specimen should be collected.

Conclusion

Regardless of how sensitive the analytical instrument used to measure trace and ulra trace element concentrations, the ubiquity of many trace elements in the environment require special precautions from pre-analytical processes through the actual analysis. If the sample collection and preparation steps are not designed to minimize contamination the results are meaningless.

References

1.Versieck JMJ. Cornelius R. Trace Elements in Human plasma or serum. Boca Raton ;FL:CRC Press. Inc.:2-68.1989.
2.Davies IJT. The clinical significance of the essential biological metals. London: William Heinemann; 1972.

3. Seiler HG. Sigel A. Sigel H.eds. Handbook on metals in clinical and analytical chemistry. New York: marcel Dekker, Inc.1994.
4.Moyer TP. Mussman GV. Nixon DE. Blood collection device for trace and ultra trace metal specimens evaluated. Clin Chem;37(5):709-714.1991.
5.Pineau A. Guiiard O. Chappius P. Arnaud J. Zawislak J. Sampling conditions for biological fluids for trace elements monitoring in hospital patients: a critical approach. Crit Rev Clin Lab Sci;30(3):203-222.1993.
6.Preparation and testing of reagent water in clinical laboratory; approved guidline-4th edition. CO 3-A4-AMD.Vol 26.No22. 2012.

7.ASTM. Standard specification for reagent waters. Document D1 193-91. Philadelphia ,PA: ASTM;1991.

8.NCCLS. Devices for collection of skin puncture blood specimens-2nd Ed: approved Guidline.1990.

9.NCCLS. Additives to blood collection devices: Heparin; Tentative Standard.1988.
10.NCCLS. Additives to blood collection devices: EDTA; Tentative Standard.1992.
11.CLSI. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition. CLSI document GP41-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
12.NCCLS. Procedures for the collection of diagnostic blood specimens by skin-puncture-Third Edition; Approved Standard. Document H4-A3. Villanove, Pennsylvania.1991.
13.NCCLS. Routine urinalysis and collection, transportation, and preservation of urine specimens; Tentative Guidline. GP16-T. Villanova, Pennsylvania.1992.
14.Channon SM. Mawhinney, Rodger RSC. et al. Accumulating aluminum deposition in bone due to aluminum hydroxide ingestion in patients with renal failure. In: Taylor A, ed. Aluminum and other trace elements in renal disease. London: Baillere Tindall. 118-122.1986.
15.Moyer, TP. Mussman, GV. Nixon DE. Blood collection device for trace and ultra trace metal specimens evaluated. Clin Chem. 37:709-714.1991.
16.Slanine P. Frech W. Ekstrom LG. Loof L. Slorach S. Cedergren A. Dietary citric acid enhances absorption of aluminum in antacids. Clin Chem.32:539-541.1986.
17.Vahter M. Lind B. Concentration of arsenic in urine of the general populationin Sweden.SCI Tot Environ. 54:1-12. 1986.
18.Nixon DE. Moyer TP. Arsenic analysis II: rapid separation and quantification of inorganic arsenic plus metabolites and arsenobetaine from urine. Clin Chem. 38:2479-2483.1992.
19.Therenod F. Lee WK. Toxicology of cadmium and its
damage to mammalian organs. Met ions life Sci. 11:415-90. 2013.
20.Stoeppler M. Brandt K. Contributions of automated trace analyses- V. Determination of Cd in whole blood and urine by electrothermal AAS..Fresenius Z. Anal Chem. 300:372. 1980.

21.Katz SA. Salem H. The biological and environmental chemistry of chromium. New York: VCH Publisher,Inc.136.1994.

22.Versieck J. Cornelius R. Trace elements in human plasma or serum. Boca Raton FL: CRC Press, Inc. 2-68.1989.

23.Versieck J. Trace elements in human body fluids and tissues. CRC Crit Rev Clin Lab Sci. 22:97-184.1985.

24.Lockitch G. Halstead AC. Albersheim S. Quigley G. Reston L. Jacobson B. Age and sex specific pediatric reference intervals and correlation for Zn, copper, selenium, iron, vitamin A and E and related proteins in normal children. Clin Chem. 34(4): 1625-1628.1988.

25.Aliasgharpour M. A review on copper, ceruloplasmin and wilson's disease.
Int J Med Invest . 4;344-347.2015.

26. Rice EW. Fenner HE. Study of the ICSH proposed reference method for serum iron assay: obtaining optically clear filtration and substitution of ferrozine. Clin Chem. Acta. 53:391-393.1974.

27.Jacobson BE. Lockitch G. Quigley G. Improved sample preparation for accurate determination of low concentration of lead in whole blood by graphite furnace analysis. Clin Chem. 37:515-519. 1991.

28.Goyer RA. Rhyne B. Phathological effects of lead. International review of experimental pathology. 12:1-77. 1973.

29.Johnson PE. Nielsen Fh. Copper, manganese, cobalt, and magnesium. Adv in meat research. 6:275-299. 1990.

30 Hurley IS.Keen CL. Manganese. In: Mertz W.ed. Trace elements in humane and animal nutrition. 5th ed, Vol 1. San Diego. Academic Press.185-223.1985.

31.Mercury. Geneva, World Health Organization.131 (Environmental Health criteria1).1976.

32.Mthyl mercury. Geneva, World Health Organization. 144 (Environmental Health criteria101).1990.

33. Berlin M. Mercury. In : Friberg L. Nordberg GF. Vouk VB. eds. Handbook on the toxicology of metals. 2nd ed. New York. Elsevier/ North Holland. 387-445.1986.

34..Abumrad NN .et al. Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. American J clin Nutrition. 34:2551-2559.1981.

35.Sunderman FW. Nickel. In: Clarkson TW. Friberg L. Nordberg GF. Sager PR.eds. Biological monitoring of toxic metals. New York: Plenum Press. 265-282. 1988.

36.Jacobson BE. Lockitch G. Direct determination of serum selenium by graphite furnace atomic absorption spectrophotometry with background correction and a reduced palladium modifier. Clin Chem .34(4): 709-714. 1988.

37.Mills CF, ed. Zinc in human biology. Berlin, Springer. Verlag. (ILSI human Nutrition Reviews). 1989.

38.King JC. Turnlund JR. Human zinc requirement . In: Mills CF, ed. Zinc in human biology. Berlin, Springer. Verlag. 335-350. 1989.