IZiNCG practical tips, 2nd Ed 2019

Collecting blood in the field for assessment of plasma or serum zinc concentration

Details on important aspects for the collection of blood for plasma or serum zinc concentration are described in IZiNCG technical brief #2 [1]. Below we provide some practical tips that maybe helpful in the field.

Precautions to prevent transmission of infectious agents when handling blood samples

All staff members have to be trained about the risks of exposure to blood borne pathogens prior to handling blood samples. Blood borne pathogens are pathogenic microorganisms that are present in human blood and can cause disease in humans. Proper procedures for collection, handling, transportation, storage, and disposal of blood samples and contaminated materials have to be followed. Page 66 of this Guideline from the Healthcare Infection Control Practices Advisory Committee (HICPAC) describes standard precautions [2].

Prevention of zinc contamination of samples

Zinc is present in serum and plasma only in very low concentrations. Thus, any contamination from external sources has to be avoided as they can dramatically change the results. The procedures described below should be followed to reduce the risk of contamination.

Practices and supplies to avoid contamination

- Regularly clean the workspace (phlebotomists' work area, laboratory work space, hood, supplies, etc). Keep it free of dust.
- The workspace should be treated like a sterile field. Contamination can come from e.g. hair, sneezes, open windows and lotions. Materials that come into contact with the sample should never come into contact with other things, e.g. keep lids upside down, do not touch pipets to the benchtop, and do not touch inside of tubes.
- Throughout blood collection and processing, only powder-free gloves should be used as the powder can be a source of zinc contamination. (Note: Gloves should always be tested for Zn-contamination prior to use; even powder-free gloves have been found to have Zn.)
- Only supplies free of zinc can be used for blood collection and sample processing. This can be achieved by purchasing certified trace-metal free supplies (if available) and by analyzing the zinc content of laboratory supplies prior to their use (see Annex 1 for list of trace-element free supplies).

Blood collection technique

- Clean subject's skin with alcohol at site of antecubital vein or the dorsal area (back of the hand)
- Restrict occlusion of subject's arm with tourniquet < 1 minute

Draw blood using stainless steel needle, and collect into trace element-free blood collection tubes (see **Annex 1** for list of recommended trace-element free supplies)

- A strong light is helpful in allowing the phlebotomist to visualize the vein. In settings without electricity, a battery-operated head lamp worn by the phlebotomist can be very useful to increase visibility.

The blood should be collected following a strict protocol that controls the time of day and fasting status of the participant. If fasting is not possible, then where feasible, each subject could be provided with a standard snack (e.g., biscuit or breast feeding) and the blood drawn after a standardized time interval (e.g., one hour).

To be able to control for these factors in the analyses, the following information should be recorded:

- Time of previous meal (including breast milk, for infants)
- Time of blood draw
- Time of centrifugation of serum or plasma
- Time of separation of serum or plasma

When drawing blood from young children, an option is to use a local anesthetic cream to reduce the pain of the blood draw. Any such product should be tested for zinc content prior to use. One product that is a vaso-dilator and found to be free of zinc is methocaine (Ametop™).

Processing samples

Once the blood is obtained, a cold chain (2 to 10 °C) must be maintained. Samples should be stored and/or transported in a refrigerator or a portable cool box (electric or with ice packs). It is recommended to keep a temperature log for temperature documentation throughout the process.

- When the storage temperature is 2 to 10 °C, the sample is 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.
- These precautions are important to prevent zinc being transferred from blood cells to serum or plasma, which leads to an artificial increase in the zinc content.
- After centrifugation, the plasma or serum should be transferred to a zinc-free polypropylene tube and stored in the refrigerator or freezer until analyses.

Aliquoting of samples in the field laboratory

To avoid external zinc contamination, we recommend that the samples should be aliquoted under a hood to protect from dust and other sources of contamination. If no laboratory hood is available, simple hoods can be built in country at low-cost. Pipettes and pipette tips used to aliquot samples should be protected from dust by storing in a zip-lock sealed plastic bag. Use of pipette tips that have not been stored in a sealed plastic bag should be avoided. Pipettes should be kept clean and, when necessary, they should be cleaned according to the manufacturer's instructions.

Centrifuge blood sample at 2000-3000 × g for 10 minutes to separate serum or plasma. If centrifuging with a portable centrifuge in the field, you need to make sure the red blood cells are being completely separated from the plasma. Discard any severely hemolyzed samples and make a note of those samples with some evidence of hemolysis; see IZiNCG Technical Brief no. 6 [3] and Hemolysis Guidance Card for practical guidance.

Store plasma or serum samples in polypropylene tubes. Avoid sample storage tubes with silicone washer seals; the seals may be a source of contamination. For long-term storage in a -20 °C or -80 °C freezer, store samples in a sealed plastic bag containing ice cubes to avoid evaporation.

Example of hoods built by carpenters (see **Annex 2**).
Example of hoods built out of plastic boxes (see **Annex 3**).

Sample analyses

Dilute sample for zinc 5 to 10-fold in solvents such as 0.05N aqueous hyperpure HCl. Read sample zinc concentration using an available instrument with appropriate standard dilutions, in-house quality controls, and Standard Reference Materials.

Examples of laboratory protocols:

- Flame atomic absorption spectrophotometry (see **Annex 4**).
- Inductively coupled plasma-optical emission spectrometry, plasma (see **Annex 5**).
- Inductively coupled plasma-optical emission spectrometry, serum (see **Annex 6**).

Reference laboratory

Inter-laboratory comparison with a reference laboratory is highly recommended. The list below is not meant to be exclusive and is not an endorsement of these laboratories.

United Kingdom National External Quality Assessment Service (NEQAS) www.ukneqas.org.uk

BIO-RAD External Quality Assurance Services (EQAS) www.qcnet.com

Statistical analyses

Prior to analysis, the distribution of plasma or serum zinc results should be reviewed to check for outliers (implausibly low or high values). One approach is to set statistically defined-cutoffs for outliers: for example, >3 standard deviation (SD) from the mean of age-specific US NHANES plasma zinc distribution [4].

Because plasma zinc concentration is reduced in the presence of inflammation, it is recommended to include the analyses of acute-phase proteins. C-reactive protein (CRP) (biomarker of acute inflammation) and a-1 acid glycoprotein (AGP) (biomarker of chronic inflammation) can be used for that purpose. When elevated acute-phase proteins are found, the corresponding zinc concentration can be adjusted statistically. Preliminary results from the Biomarkers Reflecting the Inflammation and Nutritional Determinants of Anemia (BRINDA) project suggest that correlation and decile analyses should be used to evaluate the association between PZC, CRP, and AGP concentrations before applying any statistical adjustments. For pre-school aged children, if a significant negative association between plasma zinc concentration and CRP or AGP is exhibited, application of the BRINDA regression correction approach may be supported. In women of reproductive age, the association between PZC with CRP and AGP was weak and inconsistent, suggesting that adjustment is typically not warranted.

If the time of day and time since previous meal cannot be standardized during data collection, these variables should also be controlled for during the data analysis. Please consult with a statistician for further details.

Use appropriate cutoffs depending on characteristics of study population (see **Table 1**) [5].

Table 1: Suggested lower cutoffs for serum zinc concentration (µg/dL) by age group, sex, time of day and time since last meal

Suggested lower cutoffs for serum zinc concentration (µg/dL)[1]			
Time of day and fasting status	<10 years	≥10 years	
	Males and females	Non-pregnant females	Males
Morning, fasting[2]	not available	70	74
Morning, non-fasting	65	66	70
Afternoon, non-fasting	57	59	61

[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.

References

IZiNCG. Assessing population zinc status with serum zinc concentration. IZiNCG technical brief no 2, Davis, CA, USA, 2007. Available at: https://www.izincg.org/technical-briefs/

Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Available at: https://www.cdc.gov/infectioncontrol/pdf/guidelines/isolation-guidelines-H.pdf

IZiNCG. How to deal with hemolysis for plasma/serum zinc analysis. IZiNCG technical brief no. 6, Oakland, CA, USA, 2018. Available at https://www.izincg.org/technical-briefs/

Pilch S.M. & Senti F.R. (1984) Assessment of the zinc nutritional status of the US population based on data collected in the second National Health and Nutrition Examination Survey, 1976-1980.

Brown K.H., Rivera J.A., Bhutta Z., Gibson R.S., King J.C., Lönnerdal B., et al. (2004) International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food and Nutrition Bulletin 25, S99-203.

Acknowledgments

This document was prepared by Sonja Y. Hess (University of California, Davis, CA, USA) with input from Rosalind S. Gibson and Karl B. Bailey (University of Otago, Dunedin, New Zealand), Zulfiqar Bhutta (Aga Khan University, Karachi, Pakistan) and Reina Engle-Stone, K. Ryan Wessells, Grant J. Aaron and Kenneth H. Brown (University of California, Davis, CA, USA). The revision of this document was prepared by Sonja Y. Hess (University of California, Davis, CA, USA) and Mari S. Manger (University of California, San Francisco, CA, USA) with input from Rosalind S. Gibson (University of Otago, Dunedin, New Zealand), David Killilea (University of California, San Francisco, CA, USA), and Jamie Westcott and Nancy Krebs (University of Colorado, Denver, CO, USA).

Annex 1: List of trace element-free blood collection supplies

It is important to use powder- and zinc-free gloves during each step of blood collection and blood processing. Each lot of gloves should be tested for zinc content prior to use.

Below is a list of trace element-free blood collection supplies. This list is not intended to be exclusive or an endorsement of any company.

Supplies for blood collection:

- S-Monovette for Trace Metal Analysis by Sarstedt (7.5mL; Lithium Heparin for Trace Metal Analysis; 92x15 mm); Ref # 01.1604.400
- S-Monovette by Sarstedt (7.5 ml, Clotting Activator/Serum, 92x15 mm); Ref# 01.1601.100
- Needle 21 G for metal analysis by Sarstedt; Ref # 85.1162.400
- Multifly needle with multi-adapter by Sarstedt (23 G x 3/4 in. 60 mm); Ref # 85.1640.005
- Multifly needle with multi-adapter by Sarstedt (21 G x 3/4 in. 60 mm); Ref # 85.1638.005
- Butterfly needle for infants and young children by Becton Dickinson (21G); Ref # 367284
- BD luer lock syringes with needles by Fischer; Ref # 14-826-84 (3 cc) or 14-826-84 (5 cc)

Supplies for aliquoting:

These supplies have been used successfully, but unless they are certified to be trace metal free, they should be tested for zinc content prior to use:

- SAMCO pipettes; Ref #225-1S
- Transfer pipettes by Sarstedt (3.5 mL); Ref # 86.1171
- MLR® Polypropylene pipette tips by Daigger, trace metal certified; Ref # EF20550R
- Pipette tips by Axygen; Ref # T1000-B
- Pipette tips by Bio-Rad (1-200 µl; metal free; BR-37 tips); Ref # 223-9037
- Pipette tips by Bio-Rad (200-1000 µL; metal free; BR-41 tips); Ref # 223-9041

Supplies for sample storage:

These have been used successfully, but should be tested for zinc content prior to use:

- 2-position tubes by Sarstedt; Ref #62.526.028
- 1.5 mL microcentrifuge tubes by Perfector Scientific
- 2 mL screw top tubes by Perfector Scientific
- 1.7 mL microcentrifuge tubes by Labcon; Ref # 3013 870 000
- 1.5 mL metal free micro test tubes by Bio-Rad; Ref # 223-9501
- 4 mL screw top cryovial by Fisher; Ref # 1026988E
- 2 mL screw top cryovial by Fisher; Ref # 1026988C
- 2 mL screw top cryovial by Greiner; Ref # 121263

Supplies for analysis:

- Trace metal grade hydrochloric acid by Fisher; Ref #A508-212 (for washing all glassware used in analyses)
- Zn AAS standard 1,000 ppm Zinc, Fluka Analytical, Catalog #18827 (AAS standard grade, 250 mL)
- Glycerol by Thermo Fisher; Ref # G33-500

Standard reference material:

- UTAK Serum; Ref # 66816
- NIST (National Institute of Standards and Technology) Bovine Serum; Ref # 1598a
- Seronorm™ Trace Elements Serum Level 1; Ref #201405, 3 mL

Annex 2: Example of hood built by carpenters

This is an example of a low-cost hood that was built out of wood, with glass in the front and a light for good visibility. The wood and all edges are carefully covered with a plastic wrap so that the hood can be easily cleaned with alcohol. When not in use, the hood can be closed with a removable glass.

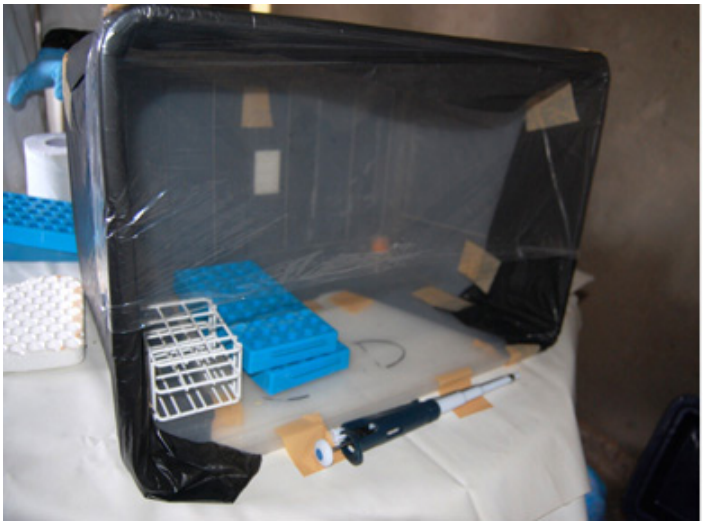
Annex 3: Examples of hoods built out of plastic boxes

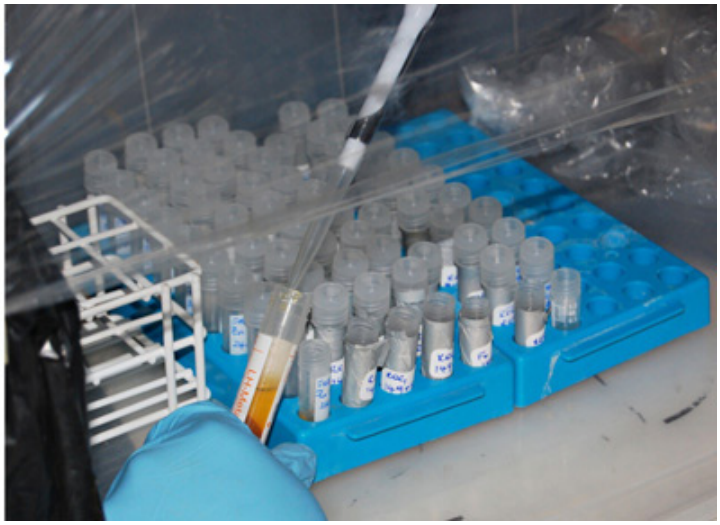
Example of a hood built out of a plastic box at very low cost

A simple storage box is positioned on its side. A plexi-glass cover was installed to cover the top half of the box to reduce the size of the opening. At the end of each day, the box can be easily cleaned with alcohol and stored with the lid closed to keep clean.

An even simpler example of a hood

A simple storage box is positioned on its side. Each day fresh cellophane foil is wrapped around the top half of the opening. At the end of each day, the box can be cleaned with alcohol and stored with the lid closed to keep clean.





Please note: Because other light-sensitive biomarkers were included in this survey, the box was wrapped with a dark cover to protect the blood samples from light. This is not necessary for many nutritional biomarkers including zinc.

Annex 4: Laboratory protocol for the analysis of serum or plasma zinc by flame atomic absorption spectrophotometry

Department of Nutrition, University of Otago, Dunedin, New Zealand

Preparation of sample diluent

The sample diluent which is used to make all standard curves and diluted samples is prepared by adding 8.34 ml of ARISTAR grade concentrated hydrochloric acid to a 2 litre volumetric flask and making up to the mark with milliQ water. (NB I used n-butanol in the past to enhance the sensitivity but because our current Atomic Absorption Spectrophotometer (AAS) is very sensitive, I have opted to exclude this component).

Preparation of standard curve

A three point standard curve is used for the determination of zinc in blood serum. First a stock solution at a concentration of 10 ppm zinc is prepared by adding 1mL from a commercial 1000 ppm Zn Standard to a 1 liter volumetric flask and made up to the mark with milliQ water. Next, 2.5 mL of HPLC grade glycerol is added to three 100 mL volumetric flasks. From the 10 ppm stock solution, three aliquots of 1.0, 2.0, and 4.0 mL are taken and each placed in one of the 100 mL volumetric flasks. Then the sample diluent is added to each flask and made up to the mark. The three flasks will now have concentrations of 0.1 ppm, 0.2 ppm, and 0.4 ppm of zinc. The glycerol is added to each flask in an attempt to match the matrix of the serum used in the controls and samples.

Preparation of serum samples and controls for analyses

A 1:10 dilution of sample or control to diluent is first carried out in order to prepare the samples and controls for analyses. To accomplish this dilution, take 0.25 mL of the sample or control and pipette into a Sarstedt trace-element free tube (part number given elsewhere in this document). Then add 2.25 mL of sample diluent to the tube. Cap the tube and mix on a vortex machine.

Note: It pays to prepare all the samples and controls first before analyses. If possible try to allow the prepared standards, controls, and samples to reach room temperature prior to analyses because temperature has a major effect on viscosity. Hence, by following this protocol, inconsistencies in the results will be minimized.

Analytical method

When doing the analysis on the AAS use the sample diluent as the blank. After the blank, analyze the standards to build the standard curve. Once the standard curve is established, then it is advisable to analyze 2 – 4 diluted pooled serum samples. The zinc concentrations of these pooled serum samples are almost always lower than expected. If this occurs, then recalibrate using the top standard of 0.4 ppm. This has the effect of lowering the standard curve. After this adjustment, the correct values are always obtained for all the controls. Normally, the analytical sequence is as follows: analysis of one control (such as UTAK) followed by an internal lab control, and then 10 prepared serum/plasma samples. Next, recalibrate with the top standard of 0.4 ppm again, and repeat the analytical sequence until all the prepared samples have been analyzed.

Note that AAS grade acetylene gas is critical in the method.

Annex 5: Laboratory protocol for the analysis of plasma zinc by inductively coupled plasma–optical emission spectrometry (ICP-OES)

David W. Killilea, Children's Hospital Oakland Research Institute, CA, USA

SAMPLE COLLECTION

1 . A . Supplies and Equipment:

- trace metal-certified or tested* blood collection device (e.g. BD # 367281, 21 gauge Saftey-Lok)
- trace metal-certified or tested* BD Vacutainer tubes (e.g. BD # 368381, K_2EDTA additive for plasma)
- trace metal-certified or tested* polypropylene 2ml microfuge tubes (e.g. Perfector Scientific #2840)
- trace metal-certified or tested* polypropylene 15ml conical tubes (e.g. Perfector Scientific #2625)
- OmniTrace 70% HNO_3 (VWR # EM-NX0407-1)
- OmniTrace H_20 (VWR # EM-WX0003-6)
- vortex mixer with a non-rubber top or barrier between rubber and sample tubes
- centrifuge and rotors capable of spinning Vacutainer & microfuge tubes

1 . B . Clinical Procedures:

1. Collect fasting venous blood according to tube instructions at standardize anatomical location and time.
2. Immediately invert whole blood 8 times to mix anticoagulant.
3. Deliver to laboratory as soon as possible kept at ambient temperature and protected from light.**

1 . C . Laboratory Procedures:

1. Centrifuge Vacutainer tubes at 800xg for 15 min at ambient temperature with no or minimum brake.
2. Pre-label two sets of microfuge tubes per patient with date, study ID, patient ID, etc.
3. Pipette ~1ml of plasma into first set of microfuge tubes, avoiding pellet or flocculent material.
4. Centrifuge microfuge tubes at 3000xg for 15min at ambient temperature with maximum brake.
5. Pipette plasma (supernatant) into second set of microfuge tubes, avoiding pelleted or flocculent material.
6. Immediately freeze microfuge tubes with clarified plasma on dry ice, then batch samples and store at -80°C.
7. Discard used blood collection devices, Vacutainer tubes, and first set of microfuge tubes in biohazard trash.
8. Send batched samples to analytical lab on dry ice, including empty tubes to check for trace metal contamination. All sample submissions should be accompanied by spreadsheet listing samples and other relevant information.

ANALYTIC DETAILS

1. Compare shipped samples to sample list. Report any concerns prior to start of sample prep.
2. Thaw plasma at room temp. Note level of apparent hemolysis using hemoglobin concentration scale.
3. If precipitate or lipid rings form, use additional centrifugation (3000xg for 15min) step as needed and avoid pellet.
4. Briefly vortex to mix sample and pipette 100µl of plasma into pre-labeled 15ml conical tube.
5. Pipette 0.25ml of OmniTrace 70% HNO3 to 'digest' sample.
6. Incubate samples overnight (16-18hrs) in acid at 60°C with 200-250 rpm orbital shaking.
7. Dilute acid lysates to 5% HNO3 with OmniTrace H20.
8. Clarify acid lysates by 3000xg centrifugation for 10 min at ambient temp, no brake.
9. Evaluate calibration curve for zinc based on standard percent errors. If values pass, continue with analysis.
10. Measure Seronorm Trace Element Serum Reference Material for zinc. If values pass, continue with analysis.
11. Measure plasma samples for zinc content using established method for zinc content in plasma.
12. Normalize elemental values to plasma volume.
13. Save residual samples for possible rerun or dilution, if needed.

NOTE

* In our experience, many tubes and supplies not designated for trace metal work may contain undetectable levels of zinc. However, unused tubes and supplies from the same lot should always be tested before use.

** If using EDTA as anticoagulant, it is important to minimize the time between draw and first centrifugation, as EDTA may promote zinc release from erythrocytes.

ANALYTIC METHODS DETAILS

- instrumentation:
  - CEM MARS5 Microwave Digestion Oven
  - Agilent 5100 SVDV ICP-OES Spectrometer
  - Agilent SPS3 Autosampler
  - PC workstation with ICPExpert 7.2
- analytic method:
  - analytes: Ag, Al, As, Au, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, In, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Si, Sn, Sr, Ti, Tl, V, Zn, & Zr
  - matrix: 5% HNO_3
  - ionization suppression: 50 mg/L Cs
  - internal standard: 5 mg/L Sc & Y
  - minimum detection limit: 5-500 µg/L, depending on element and wavelength
  - maximum detection limit: 5-50 mg/L, depending on element and wavelength

- minimum sample volume: 2.5 ml
- sample read time: 3x10 sec, mean value reported
- other operating conditions:
  - Seaspray concentric glass nebulizer type
  - RF power at 1.2 kW
  - plasma flow rate at 12 L/min
  - nebulizer flow rate at 0.7 L/min
  - auxiliary flow rate at 1.0 L/min
  - pump speed at 12 rpm

- QA/QC:
  - calibration:
    - NIST-traceable standards (various)
  - validation:
    - NIST-traceable bovine liver reference material 1577b (NIST # BLS1577b)
      - intraassay precision: BLS1577b typically <5% CV for Zn
      - interassay precision: BLS1577b typically <10% CV for Zn
    - Seronorm Trace Element Serum Reference Material (Sero # 201405 & 203105)
- spike standard recovery: 0.5 mg/L In

CONTACT

David W. Killilea, Ph.D.
Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute
CHORI Elemental Analysis Facility
5700 Martin Luther King, Jr. Way
Oakland, California 94609
dkillilea@chori.org

http://www.chori.org/Services/Elemental_Analysis_Facility/elemental_analysis.html

Updated 6/13/2016

Laboratory protocol for the analysis of serum zinc by inductively coupled plasma–optical emission spectrometry (ICP-OES)

David W. Killilea, Children's Hospital Oakland Research Institute, CA, USA

SAMPLE COLLECTION

1 . A . Supplies and Equipment:

- trace metal-certified or tested* blood collection device (e.g. BD # 367281, 21 gauge Saftey-Lok)
- trace metal-certified or tested* BD Vacutainer tubes (e.g. BD # 368380, clot activator additive for serum)
- trace metal-certified or tested* polypropylene 2ml microfuge tubes (e.g. Perfector Scientific #2840)
- trace metal-certified or tested* polypropylene 15ml conical tubes (e.g. Perfector Scientific #2625)
- OmniTrace 70% HNO3 (VWR # EM-NX0407-1)
- OmniTrace H20 (VWR # EM-WX0003-6)
- vortex mixer with a non-rubber top or barrier between rubber and sample tubes
- centrifuge and rotors capable of spinning Vacutainer & microfuge tubes

1 . B . Clinical Procedures:

1. Collect fasting venous blood according to tube instructions at standardize anatomical location and time.
2. Allow serum to clot by undisturbed incubation for 15-30 minutes at room temperature out of direct light.
3. Deliver to laboratory within 1 hour kept at ambient temperature and protected from light.

1 . C . Laboratory Procedures:

1. Centrifuge Vacutainer tubes at 800xg for 15 min at ambient temperature with no or minimum brake.
2. Pre-label two sets of microfuge tubes per patient with date, study ID, patient ID, etc.
3. Pipette ~1ml of serum into first set of microfuge tubes, avoiding pellet or flocculent material.
4. Centrifuge microfuge tubes at 3000xg for 15min at ambient temperature with maximum brake.
5. Pipette serum (supernatant) into second set of microfuge tubes, avoiding pelleted or flocculent material.
6. Immediately freeze microfuge tubes with clarified serum on dry ice, then batch samples and store at -80°C.
7. Discard used blood collection devices, Vacutainer tubes, and first set of microfuge tubes in biohazard trash.
8. Send batched samples to analytical lab on dry ice, including empty tubes to check for trace metal contamination. All sample submissions should be accompanied by spreadsheet listing samples and other relevant information.

ANALYTIC DETAILS

1. Compare shipped samples to sample list. Report any concerns prior to start of sample prep.
2. Thaw serum at room temp. Note level of apparent hemolysis using hemoglobin concentration scale.
3. If precipitate or lipid rings form, use additional centrifugation (3000xg for 15min) step as needed and avoid pellet.
4. Briefly vortex to mix sample and pipette 100µl of serum into pre-labeled 15ml conical tube.
5. Pipette 0.25ml of OmniTrace 70% HNO_3 to 'digest' sample.
6. Incubate samples overnight (16-18hrs) in acid at 60°C with 200-250 rpm orbital shaking.
7. Dilute acid lysates to 5% HNO_3 with OmniTrace H_20.
8. Clarify acid lysates by 3000xg centrifugation for 10 min at ambient temp, no brake.
9. Evaluate calibration curve for zinc based on standard percent errors. If values pass, continue with analysis.
10. Measure Seronorm Trace Element Serum Reference Material for zinc. If values pass, continue with analysis.
11. Measure serum samples for zinc content using established method for zinc content in serum.
12. Normalize elemental values to serum volume.
13. Save residual samples for possible rerun or dilution, if needed.

NOTE

* In our experience, many tubes and supplies not designated for trace metal work may contain undetectable levels of zinc. However, unused tubes and supplies from the same lot should always be tested before use.

ANALYTIC METHODS DETAILS

- instrumentation:
  - CEM MARS5 Microwave Digestion Oven
  - Agilent 5100 SVDV ICP-OES Spectrometer
  - Agilent SPS3 Autosampler
  - PC workstation with ICPExpert 7.2

- analytic method:
  - analytes: Ag, Al, As, Au, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, In, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Si, Sn, Sr, Ti, Tl, V, Zn, & Zr
  - matrix: 5% HNO_3
  - ionization suppression: 50 mg/L Cs
  - internal standard: 5 mg/L Sc & Y
  - minimum detection limit: 5-500 µg/L, depending on element and wavelength
  - maximum detection limit: 5-50 mg/L, depending on element and wavelength
  - minimum sample volume: 2.5 ml
  - sample read time: 3x10 sec, mean value reported

- other operating conditions:
  - Seaspray concentric glass nebulizer type
  - RF power at 1.2 kW
  - plasma flow rate at 12 L/min
  - nebulizer flow rate at 0.7 L/min
  - auxiliary flow rate at 1.0 L/min
  - pump speed at 12 rpm

- QA/QC:
  - calibration:
    - NIST-traceable standards (various)
  - validation:
    - NIST-traceable bovine liver reference material 1577b (NIST # BLS1577b)
      - intraassay precision: BLS1577b typically <5% CV for Zn
      - interassay precision: BLS1577b typically <10% CV for Zn
    - Seronorm Trace Element Serum Reference Material (Sero # 201405 & 203105)
  - spike standard recovery: 0.5 mg/L In

CONTACT

David W. Killilea, Ph.D.
Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute
CHORI Elemental Analysis Facility
5700 Martin Luther King, Jr. Way
Oakland, California 94609
dkillilea@chori.org

http://www.chori.org/Services/Elemental_Analysis_Facility/elemental_analysis.html

Updated 6/13/2016