

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

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**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<sub>2</sub>EDTA additive for plasma)
- trace metal-certified or tested\* polypropylene 2ml microfuge tubes (e.g. Perfeqtor Scientific #2840)
- trace metal-certified or tested\* polypropylene 15ml conical tubes (e.g. Perfeqtor Scientific #2625)
- OmniTrace 70% HNO<sub>3</sub> (VWR # EM-NX0407-1)
- OmniTrace H<sub>2</sub>O (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% HNO<sub>3</sub> 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<sub>3</sub> with OmniTrace H<sub>2</sub>O.
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<sub>3</sub>
  - **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

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