Laboratory Aspects of Trace Metal Monitoring

Ronda Greaves
Overview

- Background

- Essential Trace Elements e.g.
  - Cu
  - Zinc
  - Iron
  - Selenium

- Toxic Trace Elements e.g.
  - Lead
  - Aluminium
Definition of a trace element

A chemical element (often a metal) that is needed in minute quantities for the proper growth, development, and physiology of the organism, also known as a micronutrient.

i.e. $<1 \text{ ppm } = <1 \text{mg/L}$
### Periodic Table of Elements

<table>
<thead>
<tr>
<th>Lanthanide Series</th>
<th>Actinide Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce</td>
<td>Th</td>
</tr>
<tr>
<td>Pr</td>
<td>Pa</td>
</tr>
<tr>
<td>Nd</td>
<td>U</td>
</tr>
<tr>
<td>Pm</td>
<td>Np</td>
</tr>
<tr>
<td>Sm</td>
<td>Pu</td>
</tr>
<tr>
<td>Eu</td>
<td>Am</td>
</tr>
<tr>
<td>Gd</td>
<td>Cm</td>
</tr>
<tr>
<td>Tb</td>
<td>Bk</td>
</tr>
<tr>
<td>Dy</td>
<td>Cf</td>
</tr>
<tr>
<td>Ho</td>
<td>Es</td>
</tr>
<tr>
<td>Er</td>
<td>Fm</td>
</tr>
<tr>
<td>Tm</td>
<td>Md</td>
</tr>
<tr>
<td>Yb</td>
<td>No</td>
</tr>
<tr>
<td>Lu</td>
<td>Lr</td>
</tr>
</tbody>
</table>

#### Legend - click to find out more...

- **H - gas**
- **Li - solid**
- **Br - liquid**
- **Tc - synthetic**

- Non-Metals
- Transition Metals
- Rare Earth Metals
- Halogens
- Alkali Metals
- Alkali Earth Metals
- Other Metals
- Inert Elements

# Essential Trace Elements

<table>
<thead>
<tr>
<th>Element</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>Cofactor of many redox enzymes e.g. cytochrome c oxidase</td>
</tr>
<tr>
<td>Iodine</td>
<td>Required for the synthesis of thyroid hormones thyroxine &amp; triiodothyronine</td>
</tr>
<tr>
<td>Iron</td>
<td>Required for many proteins including haemoglobin</td>
</tr>
<tr>
<td>Manganese</td>
<td>Enzyme cofactor</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Oxidises xanthine oxidase, aldehyde oxidase &amp; sulphite oxidase</td>
</tr>
<tr>
<td>Selenium</td>
<td>Cofactor for antioxidant enzymes e.g. glutathione peroxidase</td>
</tr>
<tr>
<td>Zinc</td>
<td>Required for many enzymes e.g. liver alcohol dehydrogenase &amp; carbonic anhydrase</td>
</tr>
</tbody>
</table>
## Biological Variation Data

<table>
<thead>
<tr>
<th></th>
<th>ANALYTE</th>
<th>Biological Variation</th>
<th>Desirable Specifications</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CVw</td>
<td>CVg</td>
</tr>
<tr>
<td>S-</td>
<td>Calcium</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>U-</td>
<td>Calcium, concentration, 24h</td>
<td>27.6</td>
<td>36.6</td>
</tr>
<tr>
<td>U-</td>
<td>Calcium, ionized</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>U-</td>
<td>Calcium, output, 24h</td>
<td>26.2</td>
<td>27</td>
</tr>
<tr>
<td>P-</td>
<td>Copper</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>S-</td>
<td>Copper</td>
<td>4.9</td>
<td>13.6</td>
</tr>
<tr>
<td>S-</td>
<td>Iron</td>
<td>26.5</td>
<td>23.2</td>
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<tr>
<td>(B)Erythr-</td>
<td>Magnesium</td>
<td>5.6</td>
<td>11.3</td>
</tr>
<tr>
<td>(B)Leuc-</td>
<td>Magnesium</td>
<td>18.3</td>
<td>16.4</td>
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<td>Magnesium</td>
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<td>6.4</td>
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<tr>
<td>U-</td>
<td>Magnesium, concentration, 24h</td>
<td>45.4</td>
<td>37.4</td>
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<tr>
<td>U-</td>
<td>Magnesium, ionized</td>
<td>1.9</td>
<td>5.1</td>
</tr>
<tr>
<td>U-</td>
<td>Magnesium, output, 24h</td>
<td>38.3</td>
<td>37.6</td>
</tr>
<tr>
<td>P-</td>
<td>Selenium</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>B-</td>
<td>Selenium</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>S-</td>
<td>Zinc</td>
<td>9.3</td>
<td>9.4</td>
</tr>
<tr>
<td>P-</td>
<td>Zinc</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
Copper
Copper:

- Copper plays a key role in the development of healthy nerves, bones, collagen and the skin pigment melanin.

- Normally, copper is absorbed from food, and any excess is excreted through bile.

- Deficiencies of copper can cause premature hair greying, sterility and premature wrinkling of the skin.

- Analysis
  - Serum Copper
  - Urine copper
Wilson’s disease

- An inherited disorder that causes too much copper to accumulate in the liver, brain and other vital organs.
- Fatal if left untreated
- Autosomal recessive inheritance, many mutations spontaneous
- Mutation in gene ATP7B on chromosome 13
- >30 mutations identified currently
- Causes problem with production of ceruloplasmin, the protein that moves copper around the body.
- Impaired biliary excretion of copper results in deposition in liver → cirrhosis

Normal liver v.s. cirrhosis  liver
Wilson’s disease: signs & symptoms

- Clumsiness
- Depression
- Difficulty speaking
- Difficulty swallowing
- Difficulty walking
- Drooling
- Easy bruising
- Fatigue

- Involuntary shaking
- Joint pain
- Loss of appetite
- Nausea
- Skin rash
- Swelling of arms and legs
- Yellowing of the skin and eyes (jaundice)

http://www.mayoclinic.com/health/wilsons-disease/DS00411/DSECTION=symptoms
Zinc

Following case courtesy of
Dr James Doery - MMC
Zinc

- Zn is an essential trace element in >100 enzyme including DNA and RNA polymerase and ALP.

- Deficiencies of zinc can cause sterility, impotence and even depression

- Etiology of deficiency and appropriate treatment
  - Nutrition or inborn error of metabolism?
  - Breast milk is a rich, time dependent source of zinc especially colostrum.

- Significant diurnal variation (up to 40%)

- Zn is 65% albumin bound and will be lower if albumin low

- Measured colour change reaction or by atomic absorption
Zinc

- When should serum zinc be measured?
  - Poor wound healing
  - TPN patients
  - Typical rash (red, exudative, scaly)
  - Alopecia
  - Immune deficiency

- Sample Integrity
  - Collection tube contamination
  - Skin contamination many creams contain zinc
    - Cetamacrogol (sorbolene): NO zinc
    - Johnson’s nappy cream: lanolin, MgSO4, beeswax etc including ZnO
    - Ichthammol: contains zinc

- Unexpected results
  - If result is unexpected – question it!
  - If all else fails – look at the patient!
Case 1: Baby J – “Nappy rash with a difference” (does the lab really know what they are doing?)

- Premature infant born at 24/40
  - Chronic lung disease
  - Subglottic stenosis
  - Sepsis
  - Hypotension
  - Jaundice
  - Steroid induced hypertension
  - Gastro-oesophageal reflux
  - Retinopathy of prematurity – stage I/II
  - Bilateral inguinal hernias
  - Supraventricular tachycardia
  - Anaemia of prematurity
Case 1: - at 4 months

- Age 4 months (equivalent of term)
- Developed an extensive erythematous, exudative and scaly lesions on:
  - Perineum
  - Face
  - Hands
- This was suggestive of impetigo – a severe skin infection by staphlococci or streptococci
- Unresponsive to antibiotics
- Dermatology consult was sought and the possibility of acrodermatitis enteropathica was raised
### Case 1:

<table>
<thead>
<tr>
<th>date</th>
<th>30/10</th>
<th>1/11</th>
<th>5/11</th>
<th>6/11</th>
<th>10/11</th>
<th>RR</th>
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</thead>
<tbody>
<tr>
<td>cap</td>
<td>cap</td>
<td>ven</td>
<td>ven</td>
<td>ven</td>
<td>ven</td>
<td>RR</td>
</tr>
<tr>
<td>Zinc</td>
<td>&gt;120</td>
<td>84</td>
<td>5 &amp; 4</td>
<td>68</td>
<td>29</td>
<td>10-17 umol/L</td>
</tr>
<tr>
<td>ALP</td>
<td>68</td>
<td>230</td>
<td>80-130 U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Very HIGH zinc level was totally unexpected but also very high on repeat sampling
- Baby was not receiving any zinc supplements
- In view of past experience of mercury toxicity after extensive external application to very raw, thin or regenerating skin we enquired about zinc application.
  - “No! Only receiving Granugen cream for the rash”
- Label indicated paraffin oil base containing high level of TiO2 and ZnO.
- When a venous sample was collected on 5/11 the zinc was found to be very low!
- Zn sulphate instituted with spectacular resolution of the rash and a rise in serum zinc and ALP
Iron

Significant diurnal variation
Transferrin = transport protein
Ferritin = storage
## Case 2: Iron Studies in 14 yo female

<table>
<thead>
<tr>
<th>Test</th>
<th>Result +0</th>
<th>Results + 2 months</th>
<th>Results +16 months</th>
<th>RI</th>
<th>UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
<td>0852</td>
<td>1230</td>
<td>1400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>116</td>
<td>122</td>
<td>120 - 160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>8</td>
<td>51</td>
<td>10</td>
<td>9 - 30</td>
<td>umol/L</td>
</tr>
<tr>
<td>Transferrin</td>
<td>3.1</td>
<td>2.8</td>
<td>2.7</td>
<td>2.1 – 4.3</td>
<td>g/L</td>
</tr>
<tr>
<td>Ferritin</td>
<td>7</td>
<td>29</td>
<td>28</td>
<td>8 – 190 / 9 - 136</td>
<td>ug/L</td>
</tr>
<tr>
<td>Iron binding capacity</td>
<td>77.8</td>
<td>70.3</td>
<td>67.8</td>
<td>44.0 – 88.0</td>
<td>umol/L</td>
</tr>
<tr>
<td>% saturation</td>
<td>10</td>
<td>73</td>
<td>15</td>
<td>15 - 50</td>
<td>%</td>
</tr>
<tr>
<td>B12</td>
<td>283</td>
<td></td>
<td></td>
<td>163 - 553</td>
<td>pmol/L</td>
</tr>
<tr>
<td>Active B12</td>
<td></td>
<td>47.1</td>
<td>19 - 128</td>
<td></td>
<td>pmol/L</td>
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<tr>
<td>Red cell folate</td>
<td>988</td>
<td>660</td>
<td>633 - 1793</td>
<td></td>
<td>nmol/L</td>
</tr>
</tbody>
</table>
Selenium
Selenium

- Deficiencies can cause people to age prematurely or to have slower than normal recovery from illnesses
- Levels assessed commonly in TPN patients
- Measure in whole blood
- Atomic Absorption
- Alternatively can measure enzyme activity as a functional test e.g. glutathione peroxidase
Lead
Lead

- 1996 Australian survey – “Lead in Australian Children”

- Recommended:
  \[ <10 \mu g/dL \equiv <0.483 \mu mol/L \]

- Measure in whole blood

- Atomic Absorption
Case 3: The highest level case from the 1996 national survey of lead in children

- The 2 youngest children of a large family surveyed.

- Family situation:
  - Income - Low
  - Accommodation - Timber house built b/w 1910 & 1925
  - Water supply – from tanks that drain to a metal roof
  - Cars – 2 old cars using leaded petrol
  - Animals – dogs and cats
  - Garden – vegetable garden
  - Smoking – people smoked in the house
  - Cleanliness – rated as “dirty”
Case 3: continued

- **Results:**
  - Child 1: 3y.o.  lead = 0.51 μmol/L
  - Child 2: 15 m.o.  lead = 1.58 μmol/L

- **Investigations:**
  - With parental agreement
  - Water Tests = <5 μg/L
  - Soil sampling = 22 mg/L
  - Sandpit = 9 mg/kg
  - Isotope ratios
  - Other

- **Cause:**
  - “Pending further examination of local soils, it is considered likely that this child’s very high blood lead level resulted primarily form eating soil, with contributions from many other risk factors.”
Conclusions: “Blood lead concentrations, even those below 10µg/dL, are adversely associated with children’s IQ scores at 3 & 5 years of age.”
Aluminium
Aluminium toxicity

- Aluminium toxicity is a known hazard of ESRD
- Most abundant metal found on earth present in water and soil
- No known action in the body
- Very little is absorbed
- Readily excreted by the kidney

Toxicity associated with bone:
- Inhibition of hydroxyapatite formation
- Inhibition of bone cell proliferation
- Suppression of bone cell activity

Results in:
- Inhibition of bone mineralisation
- Decreased bone formation
- Low bone mass
Case 4: First results

- Baby boy 3 months of age
- Diagnosed with polycystic kidneys
- Rare recessive inheritance

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+</td>
<td>141 mmol/L</td>
<td>135 - 145</td>
</tr>
<tr>
<td>K+</td>
<td>4.9 mmol/L</td>
<td>3.5 - 5.1</td>
</tr>
<tr>
<td>Cl-</td>
<td>108 mmol/L</td>
<td>98 – 110</td>
</tr>
<tr>
<td>Urea</td>
<td>16.6 mmol/L</td>
<td>1.3 - 6.6</td>
</tr>
<tr>
<td>Creat</td>
<td>0.21 mmol/L</td>
<td>0.01 - 0.03</td>
</tr>
<tr>
<td>Ca++</td>
<td>2.49 mmol/L</td>
<td>1.90 - 2.70</td>
</tr>
<tr>
<td>PO4-</td>
<td>1.76 mmol/L</td>
<td>1.30 – 2.30</td>
</tr>
<tr>
<td>ALP</td>
<td>291 U/L</td>
<td>100 - 350</td>
</tr>
</tbody>
</table>
Case 4: Example results 9 years later

<table>
<thead>
<tr>
<th></th>
<th>Feb</th>
<th>Sept</th>
<th>Nov</th>
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<tbody>
<tr>
<td>Na+</td>
<td>141</td>
<td>142</td>
<td>137</td>
</tr>
<tr>
<td>K+</td>
<td>4.9</td>
<td>3.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Cl-</td>
<td>102</td>
<td>102</td>
<td>95</td>
</tr>
<tr>
<td>Urea</td>
<td>25.6</td>
<td>8.6</td>
<td>26.6</td>
</tr>
<tr>
<td>Creat</td>
<td>0.71</td>
<td>0.33</td>
<td>0.80</td>
</tr>
<tr>
<td>CRP</td>
<td>35</td>
<td>33</td>
<td>&lt;8</td>
</tr>
</tbody>
</table>

- High Aluminium result in June sample
Case 4: Aluminium

- The renal unit alerted the lab to their concern about high results for aluminium

<table>
<thead>
<tr>
<th>umol/L</th>
<th>Previous year</th>
<th>May</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>0.9</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Patient B</td>
<td>3.3plasma</td>
<td>1.9</td>
<td>6.3</td>
</tr>
<tr>
<td>Patient C</td>
<td>3.1</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Case Study</td>
<td></td>
<td></td>
<td>8.1</td>
</tr>
</tbody>
</table>

Reference Range: 0.07 - 0.56 umol/L
Attention: >2.2 umol/L
Surveillance: >3.7 umol/L
Clinical symptoms: >7.4 umol/L

From Kaplan et al methods 2009
Case 4: Aluminium

Three possible causes of error were investigated:

1. **Analytical error.**

2. **Pre-analytical error** - contamination with aluminium during sample collection.

3. **Contamination of dialysis water supply.**
Case 4: Al$^{3+}$ investigation

1. Analytical error.
   - A stored sample for Patient B collected in June was sent to an alternative laboratory for re-analysis.
   - The result was 8.6 µmol/L.
   - This confirmed the original high result of 6.3 µmol/L from the original laboratory and ruled out analytical error as the cause.
Case 4: $\text{Al}^{3+}$ investigation

2. **Pre-analytical error** - contamination with aluminium during sample collection.
   - A selection of sample collection equipment were collected from the dialysis unit for analysis of aluminium content: e.g.
     - Syringes
     - Sample collection tubes
   - The results returned demonstrated that the serum gel collection tubes were contaminated with aluminium.
   - The syringes were not contaminated.
Case 4: Al$^{3+}$ investigation

2. Pre-analytical error - continued
   - A recommendation was put forward by the analysis lab to change to an alternative tube type e.g. lithium heparin tubes.
   - A sample of the lithium heparin tubes were subsequently forwarded for confirmation of suitability for aluminium analysis.
   - The laboratory confirmed that the lithium heparin tubes are suitable for collection of aluminium samples.
Case 4: $\text{Al}^{3+}$ investigation

3. Contamination of dialysis water supply.
   - The nurse in the dialysis unit advised that the water from the dialysis unit had recently been analysed for trace metals; the results confirmed no significant contamination.

   - The unit however organised the reanalysis of their water by a different laboratory to confirm the original result post discussion.

   - This second analysis reconfirmed that the dialysis unit water did not contain unacceptably high levels of aluminium.
Case 4: $\text{Al}^{3+}$ investigation

Outcome:

- The source of the spuriously high aluminium levels was the serum gel collection tubes.

- Pathology moved to the implementation of lithium heparin tubes for future analysis of aluminium levels.
External QA program

- From 2011

RCPA QAP Chemical Pathology Program introducing a Trace Metals program
Acknowledgements for all presentations today

- Mr Rod Ward
- Prof Garry Warne
- Ms Jan Gill
- Assoc Prof Fergus Cameron
- RCPA QAP Chemical Pathology
- AACB Vitamins Working Party
- Roche Australia
- Waters Australia
Thankyou for the opportunity to return to Viet Nam and to discuss clinical biochemistry

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