Laboratory Aspects of Trace Metal Monitoring

Ronda Greaves

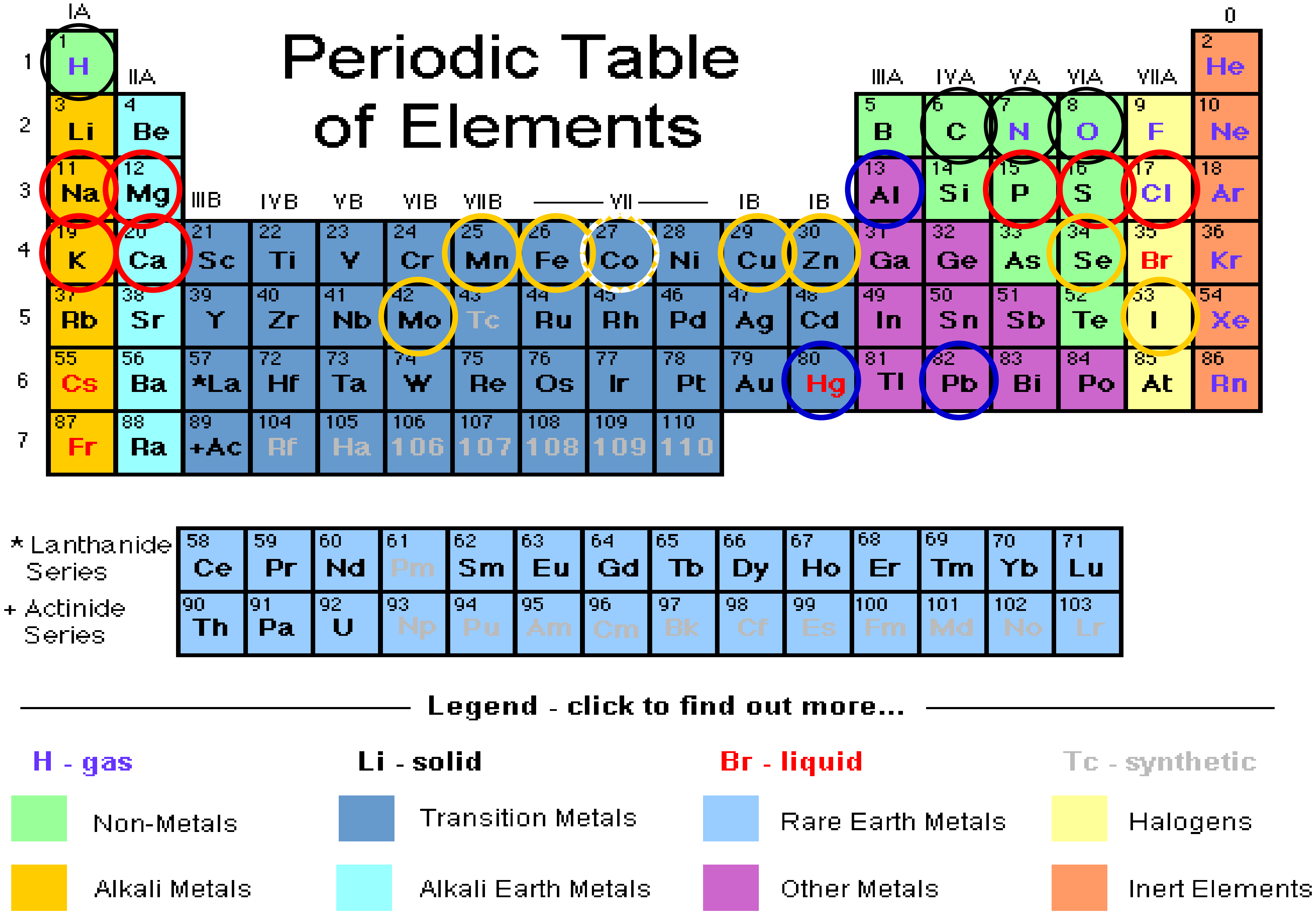
Overview

- 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 ppm = <1mg/L



http://www.corrosionsource.com/handbook/periodic/periodic_table.gif

Essential Trace Elements

Element	Use
Copper	Cofactor of many redox enzymes e.g. cytochrome c oxidase
Iodine	Required for the synthesis of thyroid hormones thyroxine & triiodothyronine
Iron	Required for many proteins including haemoglobin
Maganese	Enzyme cofactor
Molybdenum	Oxidises xanthine oxidase, aldehyde oxidase & sulphite oxidase
Selenium	Cofactor for antioxidant enzymes e.g. glutathione peroxidase
Zinc	Required for many enzymes e.g. liver alcohol dehydrogenase & carbonic anhydrase

Biological Variation Data

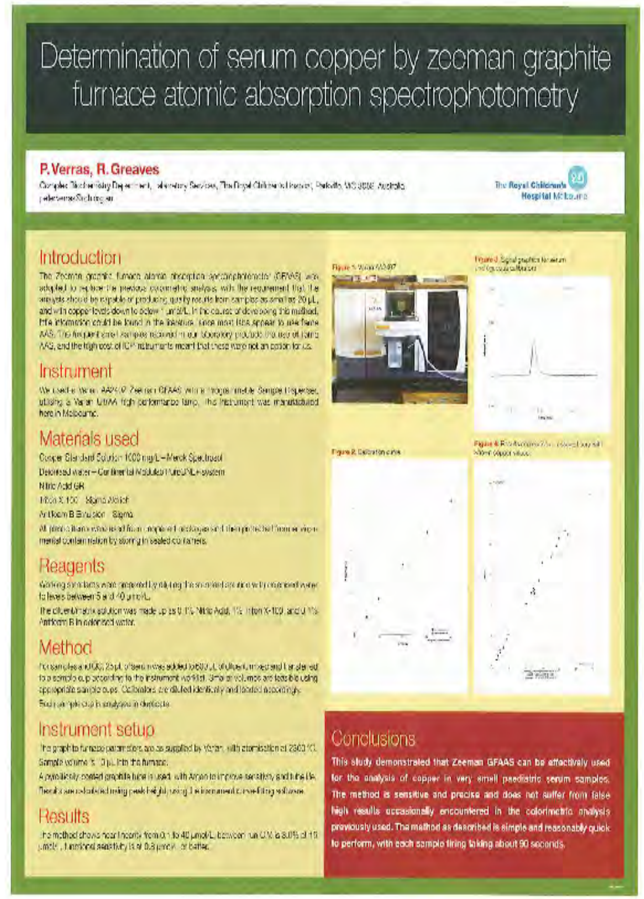
	ANALYTE	Biological Variation		Desirable Specifications		
		CVw	CVg	I(%)	B(%)	TE(%)
S-	Calcium	1.9	2.8	1	0.8	2.4
U-	Calcium, concentration, 24h	27.6	36.6	13.8	11.5	34.2
U-	Calcium, ionized	1.7	2.2	0.9	0.7	2.1
U-	Calcium, output, 24h	26.2	27	13.1	9.4	31
P-	Copper	8	19	4	5.2	11.8
S-	Copper	4.9	13.6	2.5	3.6	7.7
S-	Iron	26.5	23.2	13.3	8.8	30.7
(B)Erythr-	Magnesium	5.6	11.3	2.8	3.2	7.8
(B)Leuc-	Magnesium	18.3	16.4	9.2	6.1	21.2
S-	Magnesium	3.6	6.4	1.8	1.8	4.8
U-	Magnesium, concentration, 24h	45.4	37.4	22.7	14.7	52.2
U-	Magnesium, ionized	1.9	5.1	1	1.4	2.9
U-	Magnesium, output, 24h	38.3	37.6	19.2	13.4	45
P-	Selenium	12	14	6	4.6	14.5
B-	Selenium	12	12	6	4.2	14.1
S-	Zinc	9.3	9.4	4.7	3.3	11
P-	Zinc	11	14	5.5	4.5	13.5

http://www.westgard.com

Copper

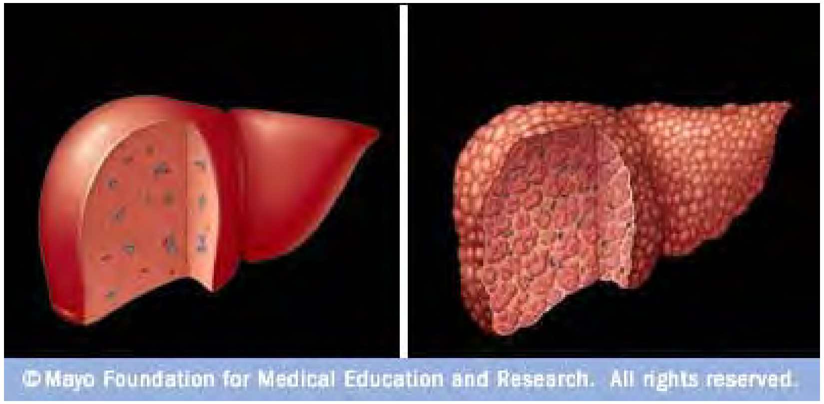
Copper:

- 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 dependant source of sinc 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, $MgSO_4$, 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:

date	30/10	1/11	5/11	6/11	10/11	
	cap	cap	ven	ven	ven	RR
Zinc	>120	84	5 & 4	68	29	10-17 umol/L
ALP			68		230	80-130 U/L

- 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 TiO_2 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

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

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 µg/dL ≡ <0.483 µmol/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."

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Intellectual Impairment in Children with Blood Lead Concentrations below 10 µg per Deciliter

Richard L. Canfield, Ph.D., Charles R. Henderson, Jr., M.A., Deborah A. Cory-Slechta, Ph.D., Christopher Cox, Ph.D., Todd A. Jusko, B.S., and Bruce P. Lanphear, M.D., M.P.H.

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

Alfrey AC, LeGendre Gr, Kaehny WD. The dialysis encephalopathy syndrome. Possible aluminium intoxication. N Engl J Med 1976; 294: 184-188.

Case 4: First results

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

	Results	RR
Na+	141 mmol/L	135 - 145
K+	4.9 mmol/L	3.5 - 5.1
Cl-	108 mmol/L	98 – 110
Urea	16.6 mmol/L	1.3 - 6.6
Creat	0.21 mmol/L	0.01 - 0.03
Ca++	2.49 mmol/L	1.90 - 2.70
PO4-	1.76 mmol/L	1.30 – 2.30
ALP	291 U/L	100 - 350

Case 4: Example results 9 years later

	Feb	Sept	Nov
Na+	141	142	137
K+	4.9	3.0	5.1
Cl-	102	102	95
Urea	25.6	8.6	26.6
Creat	0.71	0.33	0.80
CRP	35	33	<8

- High Aluminium result in June sample

Case 4: Aluminium

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

umol/L	Previous year	May	June
Patient A	0.9	1.4	1.5
Patient B	3.3plasma	1.9	6.3
Patient C		3.1	5.8
Case Study			8.1

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 umol/L from the original laboratory and ruled out analytical error as the cause.**

Case 4: 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: 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: 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

Quality Control Technologies Pty Ltd
A.B.N. 63 075 812 814

Trace Element Quality Assurance Program Result Sheet
Blood/Serum/Urine

Please note: These samples have been gamma irradiated prior to dispatch. They are of biological origin and should be handled in accordance with universal safety precautions.

- Before the "Results Due" date enter your results clearly using a black pen.
- Fax results to: (07) 3865 5392 or International: +61 7 3865 5392 and then

Post results to: Statistician, Quality Control Technologies Pty Ltd, PO Box 297 Taigum Qld 4018 Australia

Lab. Number	Round Number	Samples Dispatched	Samples Received	Results Due	Results Sent/Faxed
186	253	15/03/10	26/3/10	28/04/10	

Whole Blood Sample Number	Lead µmol/L x.xx	Cadmium nmol/L xxx	Arsenic µmol/L x.xx	Selenium µmol/L x.xx	Mercury nmol/L xxx	Manganese µmol/L x.xxx	Calcium mmol/L x.xx	Magnesium mmol/L x.xx	Potassium mmol/L xxx	Zinc µmol/L xxx
669	•		•	•		•	•	•		
670	•		•	•		•	•	•		
671	•		•	•		•	•	•		

Urine Sample Number U 207

Copper µmol/L x.xx	Cadmium nmol/L xxx	Total Arsenic µmol/L x.xx	Inorganic Arsenic µmol/L x.xx	Lead µmol/L x.xx	Mercury nmol/L xxx	Iron µmol/L x.xx	pH x.xx	Chromium nmol/L xxx	Thallium nmol/L xxx
1·76		•	•	•		•	•		

Cobalt nmol/L xxx	Antimony nmol/L xxx	Platinum µmol/L x.xx	Sodium mmol/L xxx	Potassium mmol/L xx.x	Calcium mmol/L xx.xx	Magnesium mmol/L xx.xx	Creatinine mmol/L xx.x	Aluminium µmol/L xxx.xx	Selenium µmol/L x.xx
		•		•	•	•	•	•	•

Nickel µmol/L xx.xx	Manganese µmol/L x.xx	Chloride mmol/L xxx	Fluoride mg/L xx.xx	Vanadium µmol/L xx.xx	Zinc µmol/L xx.xx	Iodine µmol/L xx.xx
•	•		•	•	•	•

Serum

Sample Number	Copper µmol/L xx.x	Zinc µmol/L xx.x	Selenium µmol/L x.xx	Aluminium µmol/L x.xx	Platinum µmol/L x.xx	Magnesium mmol/L x.xx	Manganese nmol/L xxx	Chromium nmol/L xxx	Cobalt nmol/L xxx
669	15·0	11·6	•	•	•	•			
670	13·1	12·7	•	•	•	•			

METHODOLOGY DETAILS: *Please complete Zn & Cu methodology details below.*

- From 2011
- RCPA QAP Chemical Pathology Program introducing a Trace Metals program

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New contact details

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