ICPMS-derived reference intervals for nutritional trace elements in plasma, whole blood and red cells.

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ICP-MS (INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY)

The technique of ICP-MS is now within the reach of non-specialist laboratories. It permits the simultaneous and accurate determination in blood of nutritionally important trace elements whose analysis has previously only been available in research laboratories. This is of particular importance in the case of selenium.

We have used the measurement of erythrocyte element concentrations, along with plasma concentrations, to help assess possible intracellular de-saturation of these elements in pathological states and nutritional deficiencies. This approach is especially useful in the case of magnesium, which is easily exchangeable across the cell membrane and where plasma magnesium concentrations are maintained at the expense of intracellular magnesium.

ICP-MS METHODS

Elements were simultaneously measured using a Thermo Electron XSeries II ICP-MS in CCT (reaction cell) mode, using H/He as a cell gas. The sample used was 0.25 ml of plasma diluted 1/20, or 0.10 ml of whole blood diluted 1/50, in 1% nitric acid. ^{71}Ga 50 ppb was added on line to the sample stream as an internal standard. The dwell time in the analysis programme was varied from 20-500 milliseconds, depending on the expected concentration of the element.

Performance tests were carried out on a daily basis to ensure that the ICP-MS was operating at its optimum; if the instrument failed a performance test it was re-tuned using the manufacturer's protocol. Calbration for each element was achieved through the use of a multi-element standard, diluted to three different concentrations. Both internal (UTAK Normal, UTAK High, Lyphocheck II and Seronorm) and external (TEQAS) quality control samples were used to ensure that the ICP-MS was performing to an adequate standard and with sufficient accuracy.

We found that this method was not suitable for vanadium, which was originally included in the study, due to residual interference on ^{51}V from $^{35}Cl^{16}O^{+}$. V analysis thus requires different reaction conditions and cannot easily be included in this profile as it stands.

Table 2 - Reference intervals for elements in blood

Element	2.5%	97.5%	Units
Calcium	1.10	1.73	mmol/L
Chromium	3.6	23.1	nmol/L
Cobalt	0.3	4.0	nmol/L
Copper	9.70	20.0	µmol/L
Magnesium	1.15	1.81	mmol/L
Manganese	80	200	nmol/L
Selenium	1.75	3.50	µmol/L
Zinc	64	124	µmol/L

Table 3 - Reference intervals for elements in erythrocytes

Element	2.5%	97.5%	Units
Calcium	0.40	0.80	mmol/L
Chromium	7.8	34.5	nmol/L
Cobalt	1.0	4.0	nmol/L
Copper	10.2	27.1	µmol/L
Magnesium	2.08	3.00	mmol/L
Manganese	280	760	nmol/L
Selenium	1.32	3.42	µmol/L
Zinc	190	250	µmol/L

RED CELL ELEMENT CONCENTRATIONS TO ASSESS INTRACELLULAR ELEMENTS

Venous blood samples, collected into trace element-free potassium EDTA tubes (BD Vacutainer) from 225 adult subjects (22 – 63 years of age) undergoing health screening were used as the basis for this study. Red blood cell analysis can be a useful tool for the investigation of insufficiency or excess of essential elements within cells. Red cell element levels have been used for assessing:

Muscle and cardiac effects (magnesium, calcium),
Anti-inflammatory processes (selenium, copper, zinc),
Immunological function (zinc, copper, magnesium),
Poor glucose tolerance (chromium,manganese).

Plasma reference intervals (95% interfractile interval) for ten elements are shown in Table 1. Reference intervals for calcium and iron were in agreement with those derived from colourimetric methods, while those for copper, iron, magnesium and zinc were in agreement with those derived by atomic absorption and atomic emission methods.

Whole blood reference intervals for eight elements are shown in Table 2, while erythrocyte reference intervals (calculated as the molar concentration per litre of red blood cells) are shown in Table 3.

RED CELL ELEMENT CONCENTRATIONS - calculation

The concentration of calcium, chromium, cobalt, copper, magnesium, manganese, selenium and zinc in erythrocytes was calculated by the method described by Gowenlock [1], which is based on the plasma and whole blood concentrations of the element, together with the packed cell volume (haematocrit) of the sample.

$$\text{RBC concentration} = \frac{\text{Blood concentration} - \text{Plasma concentration}}{\text{Haematocrit}} + \text{Plasma concentration}$$

Table 1 - Reference intervals for elements in plasma

Element	2.5%	97.5%	Units
Calcium	2.10	2.60	mmol/L
Chromium	6.2	33.4	nmol/L
Cobalt	0.5	8.5	nmol/L
Copper	12.5	25.0	µmol/L
Iron (female)	10.7	32.0	µmol/L
Magnesium	0.70	1.20	mmol/L
Manganese	9.0	40.0	nmol/L
Molybdenum	3.0	46.0	nmol/L
Selenium	1.00	1.90	µmol/L
Zinc	11.5	20.0	µmol/L

CONCLUSIONS

Using a "well" patient population (n = 225) we have determined reference intervals for calcium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, selenium, and zinc in plasma (from trace element-free, potassium-EDTA BD vacutainers). We have also determined reference intervals for whole blood and erythrocyte concentrations of calcium, chromium, cobalt, copper, magnesium, manganese, selenium and zinc from plasma and whole blood values, together with the measured haematocrit.

The erythrocyte concentration of nutritionally important elements may be used to help assess possible intracellular de-saturation of these elements in pathological states. This approach is especially useful for assessing magnesium deficiency, but has its limitations. For example, while functional selenium is incorporated into the erythrocyte during haematopoiesis, selenium from the plasma may be taken up by haemoglobin in the circulation as part of a de-toxification mechanism, which limits the utility of red cell selenium determination.

Calculating the erythrocyte concentrations in this way is simpler and more robust than isolating and washing the cells, which may produce falsely lowered values due to *in vitro* losses.

REFERENCES

1.Gowenlock AH. In: Varley's Practical Clinical Biochemistry, 6th Edition. Heinemann Medical Books, Oxford, 1988 pp 562.