

## Laboratory Analysis of Micronutrient in Plasma and Erythrocytes

Vitamin B1 (thiamine diphosphate; TDP) is present almost exclusively in the RBC, so vitamin B1 status was assessed by measuring TDP in RBC. A high-pressure liquid chromatography (HPLC) system with post-column ferricyanide derivatization and fluorometric detection was used. The TDP concentration in whole blood was related to the haemoglobin (Hb) in the sample (ng TDP/g Hb). The within-batch imprecision was 5.1% at 380 ng/gHb. Vitamin B2 (flavin adenine dinucleotide; FAD) measurement in whole blood and RBC was based on the method of Speek et al.(1). Briefly, whole blood or diluted RBC haemolysate was precipitated with methanol and centrifuged, and the supernatant was injected for HPLC analysis. Then, FAD was separated on an isocratic HPLC system with a reversed-phase C18 column and fluorescence detection. The within-batch imprecision for whole-blood FAD was 4.8% at 384 nmol/L and 4.8% at 2.8 nmol/g Hb RBC FAD. Vitamin B6 (pyridoxal phosphate; PLP) concentrations in the plasma and red blood cells were measured using HPLC with precolumn semicarbazide derivatization and fluorescent detection. Additionally, PLP concentrations in the red blood cells were adjusted to haemoglobin rather than to the volume of packed red blood cells because accurate pipetting of packed red blood cells is difficult due to their high viscosity. The within-batch imprecision for plasma PLP was 4.9% at 59 nmol/L and 6.3% at 16 nmol/L, and it was 5.2% at 367 pmol/g Hb for RBC PLP. Inductively coupled plasma mass spectrometry (Agilent Technologies, Cheadle, UK) was used to measure plasma zinc, copper and selenium, as well as RBC copper, zinc, selenium and magnesium, using germanium as an internal standard. The plasma was diluted ten-fold in 2% butanol, 1% ammonia, 0.05% ethylenediaminetetraacetic acid and 0.05% Triton X-100. Fifty  $\mu$ L of red blood cells were dried and then digested in 500  $\mu$ L concentrated nitric acid for 2 hours at 70°C. The coefficient of variation (CV) for all methods was less than 5%.

## Local Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory Reference ranges.

Micronutrients	Reference ranges
<b>Plasma Zn <math>\mu</math>mol/L</b>	11.0 to 18.0 $\mu$ mol/L (male, over 9 years)(2) 10.0 to 18.0 $\mu$ mol/L (female, over 9 years)(2) 10.0 to 18.0 $\mu$ mol/L (0 to 9 year)(2)
<b>Plasma Cu <math>\mu</math>mol/L</b>	1.5 to 7.0 (0 to 3 months)(3) 4.0 to 17.0 (3 to 6 months)(3) 8.0 to 20.5 (6 to 12 months)(3) 12.5 to 23.5 (1 to 5 years) (3) 13.0 to 21.5 (6 to 9 years)(3) 12.5 to 19.0 (10 to 13 years) (3) 10 to 22 (men; local lab derived) 11 to 25 (women; local lab derived)
<b>Plasma Se <math>\mu</math>mol/L</b>	0.20 - 0.90 $\mu$ mol/L (0 to 2 years)(4) 0.50 - 1.30 $\mu$ mol/L (3 to 4 years)(4) 0.70 - 1.70 $\mu$ mol/L (5 to 16 years)(4) 0.75–1.50 $\mu$ mol/L (Adults aged 17 years and over)(5)
<b>RBC Se nmol/g</b>	3.0 - 9.0 nmol/g haemoglobin (local lab derived)
<b>Vitamin B1 ng/g</b>	275 to 675 ng/g Hb(6)

<b>Vitamin B2 nmol/g Hb</b>	1.0 to 3.4 nmol/g Hb (local lab derived)
<b>Vitamin B6 pmol/g Hb</b>	250-680 pmol/g Hb (7)

## References

1. Speek A, Schaik Fv, Schrijver J, Schreurs W. Determination of the B2 vitamere flavin-adenine dinucleotide in whole blood by high-performance liquid chromatography with fluorometric detection. *Journal of Chromatography*, 228, 311-316. 1982.
2. Hotz C, Pearson JM, Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). *The American Journal of Clinical Nutrition*. 2003;78(4):756-64.
3. Lockitch G. Trace elements in pediatrics. *Journal of the International Federation of Clinical Chemistry*. 1996;8(2):46-8, 50.
4. Thomas AG, Miller V, Shenkin A, Fell GS, Taylor F. Selenium and Glutathione Peroxidase Status in Paediatric Health and Gastrointestinal Disease. *Journal of Pediatric Gastroenterology and Nutrition*. 1994;19(2):213-9.
5. Key Non Parliamentary Papers Department of H, Social C. National Diet and Nutrition Survey: Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/09 - 2011/12) (4 Volume set): Volume 1. 2015. Report No.: 1910535338;9781910535332; Contract No.: Report.
6. Talwar D, Davidson H, Cooney J, St. JO'Reilly D. Vitamin B1 status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: comparison with erythrocyte transketolase activation assay. *Clinical chemistry*. 2000;46(5):704-10.
7. Talwar D, Quasim T, McMillan DC, Kinsella J, Williamson C, O'Reilly DSJ. Optimisation and validation of a sensitive high-performance liquid chromatography assay for routine measurement of pyridoxal 5-phosphate in human plasma and red cells using pre-column semicarbazide derivatisation. *Journal of Chromatography B*. 2003;792(2):333-43.