

## **Vitamin B12**

Blood samples were frozen at -70°C in cryogenic vials. Analyses for vitamin B12 (using 300 ul sera from Year 4) were performed at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, University of Vermont, by a competitive immunoassay on the ADVIA Centaur (Bayer HealthCare, LLC) using direct chemiluminescent technology. The normal range for vitamin B12 was 72 to 1427 pmol/L and was determined from 272 serum samples. The assay CV ranges from 4% to 10% and a 6.7% CV was observed for the 5% of the sample blinded for quality control.

## **Methylmalonic acid, total homocysteine, serum 2-methylcitrate, cystathionine**

Serum methylmalonic acid (MMA), total homocysteine, serum 2-methylcitrate, and cystathionine were only tested if vitamin B12 <260 pmol/L. Vials from the same blood draw for vitamin B12 and containing 400 ul sera from Year 4 were shipped to the University of Colorado Health Sciences Center for metabolite assays of serum MMA, total homocysteine (tHcy), 2-methylcitrate, and cystathionine. They were analyzed by capillary gas chromatography-mass spectrometry (Stabler 1999). The normal ranges for the serum metabolites have been determined previously using 60 normal blood donors aged 18-65 years and were defined as 2 SD above and below the mean after normalization of the skewed data. The normal ranges are 73-271 nmol/L for MMA, 5.4-13.9 µmol/L for tHcy, 60-228 nmol/L for 2-methylcitrate, and 44-342 nmol/L for cystathionine (Johnson 2003).

Stabler SP, Allen RH, Fried LP, Pahor M, Kittner SJ, Penninx BW, Guralnik JM. Racial differences in prevalence of cobalamin and folate deficiencies in disabled elderly women. *Am J Clin Nutr.* 1999;70: 911-919.

Johnson MA, Hawthorne NA, Brackett WR, Fischer JG, Gunter EW, Allen RH, Stabler SP. Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using Title IIIc nutrition services. *Am J Clin Nutr.* 2003;77: 211-220.