**Supplemental Materials**

**Circulating pyridoxal 5'-phosphate in serum and whole blood: Implications for assessment of vitamin B6 status**

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| **Supplementary Table S1.** Key steps in sample preparation and method characteristics of PLP and TPP HPLC assays. |
|  | **Serum PLP**Immundiagnostik® (KC 2100) | **EDTA-whole blood TPP**Chromsystems® (35000) | **EDTA-whole blood PLP and TPP**Chromsystems® (52052) |
| Precipitation  | 200 µL calibrator, controls, or unknown serum samples+ 50 µL precipitation reagentVortexIncubate 10 min. at 2-8°CCentrifuge for 2 min. at 10000g | 100 µL calibrator, controls, or unknown EDTA-WB samples+ 50 µL extraction bufferMix + 150 µL precipitation reagentVortexCentrifuge for 5 min. at 9000g | 200 µL calibrator, controls, or unknown EDTA-WB samples+ 100 µL Internal Standard+ 300 µL precipitation reagentVortexCentrifuge for 5 min. at 14000g |
| Derivatisation  | 250 µL Derivatisation reagent+ 100 µL supernatant from the previous precipitation step Mix Incubate 20 min. at 60°C in water bath Place samples at 2 to 8°C for 10 min. Centrifuge for 5 min. at 10000g | 100 µL Derivatisation reagent+ 50 µL supernatant from the previous precipitation step Mix + 50 µL Derivatisation buffer+ 50 µL Stabilisation bufferIncubate 20 min. at ambient temperatureCentrifuge | 250 µL Neutralization reagent+ 100 µL Derivatisation reagent+ 250 µL supernatant from the previous precipitation step Mix Incubate 25 min. at 60°C in water bath Incubate 10 min. at 2 to 8°CCentrifuge for 2 min. at 14000g |
| Chromatography (fluorescence) wavelengths, retention time (RT) | Ext 320 / Em 415 nm, RT = 7 min. | 367 / 435 nm, RT = 5 min. | 320 / 415 nm for PLP; 367 / 435 nm for TPP, RT = 9 min. |

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| **Supplementary Table S2**. The concentrations of serum and whole blood PLP, metabolic PLP markers and creatinine in 204 samples according to subgroups by median serum PLP assay and median whole blood PLP assay.  |
|  | Reference group (both levels above the method-specific medians) | B6 insufficiency(both levels are below the method-specific medians) | Discrepancy group(serum PLP is below the median, but whole blood PLP above the median) | Discrepancy group(serum PLP is above the median, but whole blood PLP below the median) | P value |
| N | 82 | 82 | 20 | 20 | Between group |
| Serum PLP, nmol/L | 102.1 (102.4) | 15.3 (8.6) | 17.4 (7.4) | 64.8 (108.1) | / |
| Whole blood PLP, nmol/L | 215.8 (323.0) | 63.2 (14.7) | 121.0 (46.4) | 75.5 (10.6) | / |
| tHcy, µmol/L | 13.3 (7.3)1 | **17.7 (14.3)1,3** | **16.0 (10.9)** | 12.4 (2.7)3 | 0.037 |
| Cys, nmol/L | 342 (454)2 | **435 (360)2** | **916 (1394)** | 456 (396) | 0.004 |
| Creatinine, µmol/L | 74.9 (16.6) | 73.8 (18.9) | 65.0 (15.4) | 75.7 (17.2) | 0.155 |
| Data are mean (SD). The groups were compared using ANOVA test applied using the log-transformed data. When ANOVA test was significant, post-hoc Thamhane-T2 test was used to compare the insufficiency and discrepancy groups with the reference group. Median serum PLP = 30.4 nmol/L; median whole blood PLP = 86.7 nmol/L. Groups carrying the same number are significantly different. 1 p = 0.037; 2 p = 0.010; 3 p = 0.061.  |