Concentration of serum vitamin C in pulmonary diseases

D. Begovic, L. Corkovic, T. Milesevic. Clinical Health Center Dr Dragisa Mixovic, Belgrade, Serbia

Objective: Complementary/alternative medicine (CAM) is more popular than ever before. Patients with pulmonary diseases often seek so-called alternative treatment as supplementation but seldom as real alternative medicine. One of the most highly publicized, yet least understood of all the vitamins is vitamin C, also known as ascorbic acid. The lungs are constantly exposed to oxidants such as ozone and nitrogen dioxide that are inhaled or released from inflammatory leukocytes. In order to protect the lung from increased endogenous or exogenous oxidant burden, several antioxidant systems are available, including vitamin C. Recent study suggests that vitamin C is important antioxidant and indicate that taking supplemental vitamin C could benefit patients with pulmonary diseases.

Methodology: We examined the vitamin C status in 60 patients with chronic obstructive pulmonary disease (COPD) and acute pneumonia and in children with asthma. Group I included 20 patients with acute pneumonia, Group II 20 patients with exacerbation of COPD and Group III 20 patients with COPD in stable state. All 60 patients were non-smokers. Diagnosis was established by clinical, roentgenographic, laboratory and lung function examination. Vital capacity (VC), forced expiratory volume in one second (FEV1) and the ratio 100 FEV1/VC were determined. Laboratory analyses included blood leukocyte count, ESR and serum fibrinogen. Serum ascorbate concentrations were determined by spectrophotometric method (our reference values 30 - 110 µmol/L) in patients with pulmonary diseases and in control group.

Results: Spriometry in Group I patients showed: VC = 2.70±0.78(81.7%) , FEV1 =1.95± 0.34(85.8%); in Group II: VC=2.17±0.81(64.8%) ,FEV1=1.28±0.43(52.1%) and in Group III: VC=2.38±0.70(74.4%) , FEV1=1.39±0.42(56.3%). Significantly decreased VC and FEV1 (p<0.02 to p<0.01) were found in Groups I and III patients, and increased laboratory indicators of inflammation (p=0.04 to p<0.001) in Group I. Serum ascorbate concentration was significantly lower in Group I (16.02±8.51 µmol/L) and Group II (17.33±19.61 µmol/L) than in Group III (55.36±31.04 µmol/L, p<0.001). Serum vitamin C is markedly decreased in patients with acute pneumonia and in patients with COPD during exacerbation, while patients with stable COPD show normal serum vitamin C values.

Conclusions: Concentrations of serum vitamin C were not in correlation with clinical score of symptoms and/or spirometry in children with asthma. The obtained data indicate a relation between vitamin C status and COPD. Our findings are also compatible with observations that dietary vitamin C has a protective effect on pulmonary function. Higher blood levels of vitamin C may be ideal nutritional marker for overall health.

Quantification of human urine &; serum iodine by inductively coupled plasma mass spectrometry

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Background: Thyroid diseases in China are prevalent, and whether that was associated with iodine salt in their food was controversy because of lacking efficient method for the measurement of iodine. This paper aims at establishing a method for quantification of iodine in human urine and serum by inductively coupled plasma mass spectrometry (ICP-MS), and providing an assay that can be used to the evaluation of the level of iodine in routine clinical laboratory.

Method: This study was method establishment and evaluation. Ammonia, isopropanol and ultrapure water was mixed at certain ratio, and used to dilute samples in the ratio of 1:10, and then the diluted samples were analyzed by ICP-MS. Re was used as the internal standard. And linearity, lower limit of detection, recovery, precision, accuracy, carryover and stability was evaluated thoroughly. Results of iodine of pregnant women who required iodine tests were retrospectively analyzed to evaluate the status of iodine. Results of iodine in human urine and serum by inductively coupled plasma mass spectrometry were significantly higher than ICP-MS. Re was used as the internal standard. And linearity, low limit of detection, recovery, precision, accuracy, carryover and stability was evaluated thoroughly. Results of iodine of pregnant women who required iodine tests were retrospectively analyzed to evaluate the status of iodine.

Conclusion: The method only needs 30s for analysis of one sample. It was sensitive with a lower limit detection of 0.87 ug/L, the correlation coefficient was higher than 0.9999 in ten measurements. The recovery in both serum and urine was approximately 100%. Compared with NIST standard reference material 3680, the bias was less than 5% which showed that the method had good accuracy. The inter-coefficient variation (CV) for serum iodine and urine iodine was 1.2%~3.0%, 2.0%~2.9%, respectively; and total CV for serum iodine and urine iodine were 3.0%~3.8%, 4.1%~4.9%, respectively. The mean carryover of this method was 0.03% and iodine was stable for at least one month at -20°C and 4°C. The urine and serum iodine for pregnant women was 154.8±89.7 μg/L (mean±SD), 75.8±21.4 μg/L, respectively. The correlation between urine and serum iodine was 0.21. A rapid and simple ICP-MS method for urine and serum iodine measurement has been established. It is accurate and precise and can be used in the evaluation of iodine in routine clinical laboratory.

Evaluation of Serum Vitamin B12 Level in Type 1 Diabetes Mellitus

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Background: Type 1 Diabetes Mellitus an autoimmune condition is known to be associated with multiple co-morbidities. Vitamin B12 deficiency is a potential co-morbidity that is often overlooked in these patients, despite the fact that many diabetic patients are at risk for this specific disorder. Studies done on the other population have demonstrated the presence of vitamin B12 deficiency or low vitamin B12 level in Type 1 diabetes. Defining the prevalence of low or deficient serum vitamin B12 levels in the diabetic population in this part of world may aid physicians to consider screening for vitamin B12 levels in Type 1 diabetic patients and carry out further evaluations.

Methods: The cross sectional study was done by selecting 40 Type 1 Diabetes Mellitus patients from outpatient department (OPD) visiting endocrinology unit in Kanti children’s Hospital. 30 healthy control groups were also selected based on inclusion/exclusion criteria. Serum C-peptide, vitamin B12, creatinine, blood sugar level were assessed along with glycosylated hemoglobin in both groups. SPSS ver. 22 was used to analyze the data; t-test and one way ANOVA were used to find mean differences and Pearson’s correlation was used to establish the correlation.

Results: The mean age of Type 1 Diabetic patients was 10.44 ± 3.68 years, which included 21 male and 19 female patients. Among 40 patients, 47% were diagnosed before 1 year and 53% of them were diagnosed for more than 1 year. A total of 30 controls were also included in the study, males and females being equally represented. The mean age of control group was 4.87 ± 3.53 years. The age ranged from 1 year to 14 years. The case and the control group did not differ in biochemical and demographic characteristics except in their age; the difference in age was statistically significant. The mean serum vitamin B12 level of the case was 280.37 ± 111.34 pg/mL. Among the population 40.0% i.e. 16/40 were found to be deficient and 37.5% i.e. 15/40 were sub clinically deficient. Whereas the mean serum vitamin B12 level of the control group was 462.67 ± 184.32 pg/mL. 2 out of 30(6.7%) were deficient, 8 out of 30 (26.75%) were found to be sub clinically deficient. Significant difference was noticed in the mean serum level of vitamin B12 between two groups.

Conclusion: This study demonstrated the presence of low serum vitamin B12 levels in Type 1 Diabetics. The routine screening for this condition along with confirmatory test and detail clinical examination could benefit the Type 1 diabetic patients. However, further studies on a larger population using additional markers to investigate the actual cause of deficiency are must to strengthen this statement.

Evaluation of Serum Vitamin B12 Reference Intervals According to Different Age Groups on 2 Immunoassay Platforms

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Background: Vitamin B12 deficiency is common in the older population due to gastrointestinal malabsorption, inadequate nutritional intake, increased cobalamin demand or the use of certain medications, and may be related to cognitive impairment,
mood and psychotic disorders. The cut-off limits of 350 or 400 pg/mL for sufficiency were proposed by different studies. Raising clinician awareness in order to accurately diagnose and treat vitamin B12 deficiency can prevent irreversible structural damage and reduce morbidity among elderly patients.

**Methods:** In our study, we aimed to determine the age specific parametric reference intervals for DXI (competitive binding immunoenzymatic assay; Beckman Coulter, USA) and modular E (electrochemiluminescence immunoassay; Roche Diagnostics, Germany) for the evaluation of method performances in detecting insufficiency and the number of patients requiring treatment, using hospital data (Beckman-Coulter data; between January-July 2015 and Roche data; between July 2013-June 2014; n=75136) of Marmara University Pendik E&R Hospital.

**Results:** Data were analyzed in accordance with CLSI EP28-A3C guideline on Defining, Establishing, and Verifying Reference Intervals In The Clinical Laboratory. The reference ranges were significantly different in all age groups for the two instruments (Independent Samples T-test; P<0.05) (Table 1). In our adult population, 72% of patients analyzed by Beckman-Coulter and 46% of patients analyzed by Roche were below the 300 pg/mL limit of insufficiency and 87% of patients analyzed by Beckman-Coulter and 70% of patients analyzed by Roche were below the 400 pg/mL threshold of treatment.

**Conclusion:** Clinicians should be aware that reference ranges might change considerably between different platforms and affect the number of patients determined as insufficient. Reliance on reference ranges which have a low sensitivity and specificity in diagnosing deficiency states may lead to miss this treatable condition.

Table 1. Serum vitamin B12 reference ranges on Beckman-Coulter and Roche Immunoassay platforms

<table>
<thead>
<tr>
<th>Age interval (n)</th>
<th>DXI (Beckman Coulter)</th>
<th>Modular E (Roche)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 y (n=778)</td>
<td>101-727</td>
<td>339</td>
</tr>
<tr>
<td>0-1 y (n=390)</td>
<td>106-693</td>
<td>347</td>
</tr>
<tr>
<td>0-1 y (n=388)</td>
<td>94-790</td>
<td>331</td>
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<tr>
<td>1-12 y (n=3645)</td>
<td>147-663</td>
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<tr>
<td>12-18 y (n=3837)</td>
<td>145-651</td>
<td>336</td>
</tr>
<tr>
<td>13-18 y (n=2001)</td>
<td>109-457</td>
<td>231</td>
</tr>
<tr>
<td>13-18 y (n=1184)</td>
<td>110-447</td>
<td>228</td>
</tr>
<tr>
<td>19-70 yrs (n=34995)</td>
<td>116-566</td>
<td>269</td>
</tr>
</tbody>
</table>

**A-395**

Direct validation of an analytical procedure for determination of β-carotene in human serum by UPLC-UV

R. A. Munaira, F. B. Pereira, L. A. D. Mendes, L. F. Maia, E. Mateu, A. C. M. Vidal, Hermes Pardini Institute (Toxicology Sector), Vespasiano, Brazil, Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil

The validation of analytical methods is a procedure aimed at ensuring the quality and reliability of a test. Also aims to ensure compliance with the requirements nationally and internationally accepted and verifying the adequacy of the method it intends to use. β-carotene is a carotenoid that, once ingested, can be converted into Vitamin A (retinol) or act as an antioxidant to help protect cells from the damaging effects of free radicals. The aim of this study was to perform direct validation of procedure for determination of β-carotene in human serum by UPLC-UV. Initially, there was prepared a standard solution of β-carotene in dichloromethane with a concentration of 500.0 mg/mL. An intermediate solution was prepared in ethanol with a concentration of 20.0 mg/mL, to be tested points related to the analyte calibration curve. The points tested had the following concentrations: 50.0 ng/mL, 100.0 ng/mL, 300.0 ng/mL, 500.0 ng/mL, 850.0 ng/mL, 1000.0 ng/mL, 2000.0 ng/mL and 3000.0 ng/mL. Apart from this curve, a serum pool was contaminated to obtain the same concentrations above. Among these parameters, linearity, accuracy, limits of detection and quantification and matrix effects were evaluated for consolidation of the procedure. The calibration curves for all compounds were linear with r² > 0.9996. The linear analytical range of the procedure was between 50.0 and 2000.0 ng/mL. Accuracy (92.87-111.71%), intra-assay precision (0.75-10.77%) and inter-assay precision (4.26-16.45%) were acceptable. The determination limit was 10.0 ng/mL and the quantification limit was 50.0 ng/mL. Based on the slopes of the aqueous and biological matrix curves, F-test and T-test, it was concluded that the method is nonexistent in the present study. In conclusion, the UPLC-UV method has been developed successfully for quantitative analysis of β-carotene in clinical routine.

**A-396**

Comparison of a LOCI Vitamin D Total Assay® on the Dimension EXL System to CDC-certified Assays

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**Background:** Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The Vitamin D Standardization Program (VDSP) was created to establish a standard for accurate and comparable results for the detection of 25(OH) vitamin D across laboratories. As part of the VDSP, the CDC Vitamin D Standardization-Certification Program (VDSCP) enables laboratories to earn certification based on passing four consecutive quarterly sample challenges that assess bias and imprecision. The goal of this study was to investigate the relationship between a LOCI® Vitamin D Total assay® on the Dimension EXL™ Integrated Chemistry System under development by Siemens Healthcare and current VDSP-certified vitamin D assays.

**Methods:** 101 samples purchased from various specimen vendors and sent to the (University of Ghent) to have values assigned from the ID-UCMS/MS reference method were tested using the Dimension EXL, ADVIA Centaur® XP, Roche cobas e 411, and DiaSorin LIAISON vitamin D assays. The range of the reference method values for the samples was 9.3 to 70.2 ng/mL. Results that were outside the analytical measurement range of one or more assays tested or the VDSP measurement range of 9 to 110 ng/mL were excluded. Passing-Bablok regression analysis was performed to assess the relationship between each method and the Dimension EXL assay, and all assays to the reference method, as recommended by CLSI EP-9A3.

**Results:** The Dimension EXL Vitamin D Total assay demonstrated slope within ±10% of the Roche, ADVIA Centaur, and DiaSorin vitamin D assays. Additionally, all assays demonstrated slope within ±5% to the reference method, as shown in the table.

**Comparison to Dimension EXL Assay**

<table>
<thead>
<tr>
<th>Platform</th>
<th>Slope</th>
<th>Intercept (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche cobas e 411</td>
<td>1.10</td>
<td>0.04</td>
</tr>
<tr>
<td>DiAsorin LIAISON</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ADVIA Centaur XP</td>
<td>1.09</td>
<td>2.50</td>
</tr>
</tbody>
</table>

**Comparison to ID-UCMS/MS**

<table>
<thead>
<tr>
<th>Platform</th>
<th>Slope</th>
<th>Intercept (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche cobas e 411</td>
<td>0.95</td>
<td>0.47</td>
</tr>
<tr>
<td>DiAsorin LIAISON</td>
<td>1.05</td>
<td>-0.13</td>
</tr>
<tr>
<td>ADVIA Centaur XP</td>
<td>0.95</td>
<td>-0.36</td>
</tr>
<tr>
<td>Dimension EXL</td>
<td>1.05</td>
<td>0.51</td>
</tr>
</tbody>
</table>

**Conclusion:** The Dimension EXL Vitamin D Total assay demonstrates acceptable correlation to the vitamin D reference method procedure and methods that have obtained VDSP certification.

*Under development. Not available for sale. Due to local regulations, not all products will become available in all countries.

**A-397**

Serum Transferrin Receptor- Ferritin Index as a Marker of Iron Deficiency Anemia in Active IBD patients in Indian Population

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**Background:** Inflammatory bowel diseases (IBDs)are Crohn’s disease(CD) and Ulcerative colitis(UC).Anemia in IBD is most common systemic complication.

Tuesday, August 2, 9:30 am – 5:00 pm
Methods: This is a retrospective study of 480 patients of IBD, attending OPD in Asian Institute Of Gastroenterology Hyderabad, India from Dec 2013-Dec 2015 Group I consists of CD(n=260),M:F160:100,LUC(n=220,M:F120:100), age groups (0-70)years, which were divided into Subgroup-A 0-12(Children),Subgroup-B(12-18) Adolescents,Subgroup-C(18-70) Adults, were diagnosed on Clinical,Endoscopic, Histological, Radiological and Biochemical findings, were compared with age and sex matched Group II healthy controls (n=200 M: F 120:80).For all investigations of HS cp, Stool for fecal calprotectin, Hemoglobin, S.Ferritin(SF), Vit B12,B9, LDH, Transferrin Saturation index(TiBC),and sTfR were done. sTfR levels were measured by Immunoturbidimetry On Cobas e601.S.Ferritin were measured by Electrochemiluminescene on Cobas e601Roche Diagnostics. tSfR-F index was calculated on the ratio:sTfR/Log Ferritin. Criteria for IIA was ferritin<30ng/ml, transferrin saturation <15 %and sTfR-F<2.0 in the presence of inflammation.

Results:

In study I: Group I patients were compared with Group II controls which showed 320 (66.5%)patients had active disease which were assessed by inflammatory markers, in that 280 (58.3%) patients had CD(n=182) 65.0% and UC(n=98)12.1% other colitis(n=8) 2.9 % diagnosed as active. In Study II: Group IIB patients are subgrouped as Subgroup I: 158 (35.4%) patients had IIA. Subgroup II: 16 (7.3%) patients had ferritin between 30-100ng/mL (mixed IIA/ACD) SubgroupIII:66 patients (23.3%) had ferritin >100ng/mL (ACD) and Subgroup IV:10 (5.6%)patients had Vit B12 and B9 deficiency excluding sTfR-F analysis. In Study III Patients in Subgroup II again subdivided in the presence of inflammation, to identify IIA with sTfR-F index as Group A: 28 of 46 patients (60.8%) had sTfR-F index >2, Group B: 15 patients (32.6%) had sTfR-F index =1- 2, and Group C: 3 patients (6.2%) had sTfR-F index <1.Initially only Subgroup I was diagnosed as IIA(56.4%), but with sTfR Index additional patients in GroupA, has increased IIA by 66.5% so Overall IIA was diagnosed in 186 of 280patients (66.5%),but 64 (23.3 %) of 158 diagnosed with IIA did not have anemia.In Study IV: IIA cases, sensitivity of sTfR-F index was100%, sTfR 89% and SF 85%.Specificity of sTfR and sTfR-F Index were 80.60% >SF which has lower specificity 73.90%. In ACD sensitivity sTfR-F index & sTfR is 89.80% SF 81.80%. So specificity sTfR is 100%, sTfR-F index 97.20%, SF 77.80%. In Study V: In IIAA statistical significance (p < 0.0001) was seen in female compared to male, and in children when compared to adolescence and adults with sTfR-F index

Conclusion: Our study suggests sTfR-F index to be very efficient and positive marker ,So can be an early diagnostic marker, to improve the diagnosis of IIA in IBD patients.

A Lumipulse G Assay for Quantitation of 25-OH Vitamin D in Human Serum and Plasma

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Background: Circulating 25-OH vitamin D is the indicator of vitamin D status in human body and the major storage form of vitamin D in blood (Holick MF, 2009). 25-OH vitamin D assays are required to measure both 25-OH vitamin D2 and 25-OH vitamin D3.

Methods: The Lumipulse G 25-OH Vitamin D assay is a Chemiluminescent Enzyme Immunoassay for the quantitative determination of 25-OH Vitamin D2 and 25-OH Vitamin D3 in human serum and plasma on the Lumipulse G1200 System via a twostep sandwich immunoassay method using two monoclonal antibodies against 25-OH vitamin D. The amounts of 25-OH vitamin D in specimens are obtained from the luminescence signals derived from the substrate AMPPD (3(2‘-aiproadamantane)-4-methoxy-4-(3‘-phosphoryloxy)phenyl-1, 2-dioxetane disodium salt).

Results: The Lumipulse G 25-OH Vitamin D assay demonstrated linearity from 6.0 to 150.0 ng/mL for serum and plasma, and an analytical sensitivity with the LoQ (Limit of Quantitation) ≤ 4 ng/mL. The precision study of 3 controls and 5 sera (n = 120 for each sample) revealed a total %CV ≤ 8.4% at 3 testing sites using 3 lots of reagents. There was no High Dose Hook effect with up to 3,000 ng/mL of 25-OH vitamin D2 and 25-OH vitamin D3 in samples. Equimolarity study showed a recovery within 100± 10% for the pooled serum samples with varying amounts of 25-OH vitamin D2 and 25-OH vitamin D3 in 7 different ratios, and with a targeting final concentration of 50 ng/mL. Interference studies demonstrated an average percent difference ≤ 10% between control and test samples for potential interferents, including 13 endogenous substances (human anti-mouse antibody, rheumatoid factor, conjugated bilirubin, unconjugated bilirubin, human gamma globulin, biotin, triglycerides, hemoglobin, human serum albumin, uric acid, cholesterol, L-aspartic acid and human vitamin D binding protein) and 22 drugs, which were spiked individually into sera (test samples). Cross-reactivity study revealed a ≤ 2% cross-reactivity with each 100 ng/mL 3-epi-(25-OH) vitamin D2, 100 ng/mL 3-epi-(25-OH) vitamin D3, 20,000 ng/mL vitamin D2, 20,000 ng/mL vitamin D3, 8,000 ng/mL 1α-OH vitamin D3 and 25 ng/mL Paricalcitol, and ≥ 21% cross-reactivity with each 1,25(OH)2 vitamin D2, 1,25(OH)2 vitamin D3 and 24,25(OH)2 vitamin D3 at 100 ng/mL, respectively. A comparison of Lumipulse G 25-OH Vitamin D with the predicate device, LIASON 25 OH Vitamin D TOTAL, was analyzed using weighted Deming regression. The slope and correlation coefficient (r) obtained were 1.09 and 0.9476, respectively, for the tested specimens (n = 137) which ranged from 4.0 to 107.3 ng/mL. A comparison of Lumipulse G 25-OH Vitamin D with the CDC Reference Method, ID-HPLC-MS/MS 25-OH Vitamin D was also similarly analyzed. The slope and correlation coefficient (r) obtained were 0.97 and 0.9986, respectively, for the tested specimens (n = 119) which ranged from 5.8 to 149.0 ng/mL.

Conclusion: The Lumipulse G 25-OH Vitamin D assay has demonstrated to be accurate, precise, and sensitive for the quantitative and equimolar determination of 25-OH vitamin D in human serum and plasma.

A-399

Development of a One-Step HPLC Method for Simultaneous Quantitation of Vitamin B6 and Its Metabolite 4-Pyridoxic Acid in Plasma and Serum

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Background: Vitamin B6 deficiency is associated with a wide spectrum of symptoms and can cause seizure in infants. Pyridoxal 5-phosphate (PLP), the biologically active form, is a valid indicator of vitamin B6 status. Concurrent measurement of the final metabolite 4-pyridoxic acid (PA) provides additional information regarding supplement intake and hypophosphatasia. The aim of this study is to develop a simple method that simultaneously detects PLP and PA.

Methods: A reverse phase HPLC method with fluorescence detection was optimized by comparing different derivatization, columns, mobile phases, and calibrations. The optimized method was evaluated and data was analyzed using EP Evaluator software.

Results: Procedure and typical chromatogram were shown in figure 1. Pre-column derivatization using semicarbazide showed best performance in terms of signal to noise ratio, retention time and peak shape when compared to pre- or post-column derivatization with chlorite, pre-column or in-mobile phase derivatization using sodium bisulfitie. C18 50mm-columns with 2.7um core-shell and 1.8um particles achieved baseline separation for both PLP and PA, while 4.6um particle could not resolve PA with its adjacent peaks. When compared to calibration using 5-level external standards, adding 4-deoxypyridoxine as internal standard did not improve precision or accuracy. The method became less robust to column pressure variation because of a timely switch in excitation and emission wavelength between the closely eluting 4-deoxypyridoxine and PLP peaks. The analytical measurement range was 7.7-300 nmol/L and 3.7-300 nmol/L for PLP and PA respectively. The total imprecision was below 15% for PLP and 5% for PA. The spike recovery of PA was 94.2±4.6%. Method comparison of PLP with a reference laboratory showed correlation coefficient 0.9845, slope 1.070, intercept -3.54 and mean bias 2.39nmol/L (n=43).

Conclusion: This method combines derivatization and protein precipitation in one step. It is simple and reliable for routine evaluation of vitamin B6 status.
Efforts to Control Physician Ordering of Serum 25-Hydroxy-Vitamin D in a Community Setting Have Failed to Prevent Unnecessary Testing

A. Ptolemy, M. Godwin, G. Waite, D. Bailey, H. Li. Dynacare, London, ON, Canada

Background: In 2010, the Ontario Health Technology Advisory Committee (OHTAC) in Ontario Canada recommended that routine serum 25-hydroxy-vitamin D (vitamin D) testing not be performed, except for patients with osteoporosis, rickets, osteopenia, malabsorption syndromes, and renal disease or those taking drugs that affect vitamin D metabolism. If a patient meets one of these eligibility requirements, the Ontario Health Insurance Plan (OHIP) will pay for this testing. If they do not, the patient is responsible for payment. On December 1, 2010 a new OHIP laboratory requisition was issued requiring clinicians to classify the patient as insured or uninsured for the ordered vitamin D test. In the fiscal year 2011/2012 OHIP insured vitamin D test volume decreased relative to the previous year. The OHTAC noted that they expected this trend to continue, however, this expectation has not yet been verified in a published follow-up study. Objective: Assess the impact of the OHTAC recommendations and OHIP laboratory requisition changes on the frequency of vitamin D tests performed by our regional reference laboratory and characterize the vitamin D status of our patient population. Methods: All vitamin D test orders from July 2005 to July 2015 were extracted from our laboratory information system. Patient vitamin D status was determined using the respective definitions: deficiency, <25 nmol/L; insufficiency, 25 to 75 nmol/L; sufficiency, 75 to 250 nmol/L; and toxicity, >250 nmol/L. Results: N=1,137,526 (N=338,350 male, N=799,176 female; median age 56 y; age range 1 to 101 y) insured vitamin D tests were performed from July 2005 to July 2015. Monthly volume increased exponentially from N=884 tests in July 2005 to a peak of N=30,533 tests in March 2010. The new OHIP laboratory requisition was introduced in December 2010 and that month’s insured vitamin D testing volume (N=2,143) was 89% less than the test volume of November 2010 (N=19,117). Following this requisition launch, insured vitamin D test volume grew an average of 3.8% per month. The prevalence of vitamin D insufficiency consistently trended downward from 62% of the patient population in July 2005 to 49% in July 2015. Vitamin D sufficiency prevalence, however, consistently trended upward from 36% in July 2005 to 50% in July 2015. Since April 2012, the monthly prevalence of vitamin D deficiency in our patient population was <1.5%. The monthly prevalence of vitamin D toxicity did not exceed 1% over the studied time period. Conclusion: Mandating ordering physicians to classify vitamin D insurance status on the laboratory requisition in December 2010 did initially reduce insured test volume; however, subsequently insured test ordering has consistently increased month-to-month. The OHTAC vitamin D test utilization recommendations have not changed and this relative increase is not justified by the observed trends in vitamin D sufficiency and insufficiency levels within our patient population. The OHTAC and other laboratories may use this study to review their institutions current policies on vitamin D test utilization and potentially mitigate undue financial burden to their health-care systems through inappropriate ordering practices.