Objectives: Vitamin K is an essential cofactor in the synthesis of active blood-coagulation factors, vitamin K prevents bone loss and fractures. Objective of this study was to evaluate new HPLC method for determination of vitamin K, and vitamin K in human serum.

Methods: We developed a new HPLC method for the determination of vitamin K and vitamin K in human serum with fluorescence detection after post-column zinc reduction. Vitamin K and K, were purchased from Sigma-Aldrich. The internal standard was obtained from Immundiagnostik AG, Germany. 20 μL of internal standard were added to 500 μL of serum and 2 mL of ethanol were added to precipitate the proteins. The mixture was extracted with 4.0 mL of hexane for 10 min and than centrifuged at 3000 rpm for 5 min. The lower layer was additionally re-extracted with further 4 mL of hexane. The organic layers were than evaporated at 50 °C under a stream of nitrogen. The dry residue was reconstituted with 2 mL of hexane and 0.2 mL of methanol, 100 μL acetonitrile, 1.1 g zinc acetate, 10 mL acetic acid and 10 mL water, flow rate 1.0 mL/min. The retention times were 5.7 min, 9.2 min, 10.8 min for vitamin K, internal standard, vitamin K, respectively.

Results: A linear relationship between serum concentration and peak area was obtained for both substances with correlation coefficient r²=0.9959 for vitamin K, and r²=0.998 for vitamin K. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis and coefficients of variation were less than 8%. Mean recoveries of the corresponding compounds were 94.5% and 104%. No interference has been found between vitamin K, and vitamin K, or IS.

Conclusions: The analytical method developed to quantitate vitamin K, and vitamin K, in serum has been succesfully validated.

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organisation 00064203 (University Hospital Motol, Prague, Czech Republic)
Evaluation of Vitamin D Levels in Routine of a Private Laboratory


Background: Humans get vitamin D from exposure to sunlight and from their diet, metabolized in the liver to 25-hydroxyvitamin D, which is used to determine a patient’s vitamin D status. Many studies considered major health problems from vitamin D deficiency resolved. But vitamin D deficiency is common in dark-skinned persons living in northern countries, or in Asian countries for example. This study tries to elucidate the prevalence of vitamin D deficiency and the geographic influencing factors in population of Brazil.

Objective: Compare serum concentrations of vitamin D (25-hydroxyvitamin D) in samples analysis in a large laboratory, with nationwide coverage in some periods of the year representing different sun exposures.

Methods: We use a cross-sectional study in a sample of patients who performed the dosage of 25(OH)D in a private laboratory in Brazil. We analyzed 19,185 samples of both genders aged ≥18 years. Two months were selected for the study: January and July 2012, representing the months of highest and lowest incident of sunlight, respectively.

The study was divided empirically into three geographic regions of different latitudes, and different exposures to sunlight, designated as Region (0): latitudes greater than -10°, Region (1): latitudes between -10° to -20°, and Region (2) latitudes lower than -20°.

Results: The samples collected in July showed concentrations of Vitamin D lower than those collected in January, with a higher proportion of sample with values considered deficient (<20ng/mL). The difference was statistically significant.

The proportion of deficient patients in January and July respectively was 15.51% and 25.50% with statistically significant difference. The chance of being deficient was 1.87 times higher for samples collected in July (odds ratio: IC95%: 1.73-2.01; p<0.0001).

There were differences in the percentage of deficient patients in Region (2) between months of January (17.42%) and July (30.25%). Other studies have found low levels of 25(OH)D in 42% of seniors in São Paulo and mean serum levels of 21.4ng/mL in Rio Grande do Sul, both states of Region (2).

Conclusion: The role of vitamin D deficiency in increasing the risk of many common and serious diseases, including some common cancers, type I diabetes, cardiovascular disease, and osteoporosis. The study confirms that the concentrations of Vitamin D are much lower when derived from regions of lower latitudes and during the month of July (compared to January) possibly due to a reduction in the incidence of sunlight. That conclusion should be taken in consideration and included in the clinical analysis of the patient and not the test result only itself.

Food-specific IgG antibodies in Brazilians: a descriptive, laboratory information management system-based study.


Background: The aim of this study was to identify the most common foods that trigger IgG immune responses in Brazilians of different age groups and describe the prevalence of this food hypersensitivity for the most common commodities available for consumption.

Methods: We retrieved the results from our food-specific IgG antibodies test performed between December 2012 and December 2013, 281 individuals (unique) were included and 185 (65.8%) were female. The five most common foods that trigger moderate (24-30 U/mL) or strong (>30 U/mL) IgG antibody responses were analyzed and presented by age group. The same was done with the commodities. The age groups were 1-10 (n=34), 11-20 (n=33), 21-30 (n=33), 31-40 (n=33), 41-50 (n=42), 51-60 (n=49), 61-70 (n=29), 71-80 (n=9). The food-specific IgG assay was Genarray Microrayy (Genesis Diagnostics), which detects total IgG against 221 foods in serum by ELISA.

Results: The five most common foods that trigger IgG reaction in the patients were wheat (96.1%), cola nut (84.7%), cow’s milk (75.4%), egg white (70.1%) and yeast (64.8%) and their age-dependent relative occurrence frequency can be found in figure 1A. Considering the commodities, their relative occurrence frequencies were as follows: wheat (described before), bean (57.7%), maize (40%), rice (17.1%), cassava (9.3%), pigment (2.1%), bovine meat (0%) and poultry meat (0%). Excepting the younger, each commodity elicits a constant and specific pattern of IgG hypersensitivity over age (figure 1B).

Conclusions: The most common foods that trigger moderate/strong IgG response in Brazilians and also the hypersensitive frequency for the major commodities. Some foods (Cola nut, cow’s milk, egg white and brewer’s yeast) showed an age-dependent IgG serum levels that correlates with an expected age-dependent consumption. Conversely, the constant and specific patterns found for commodities suggest that these foods elicit specific immune response, especially when confronting the extremes (wheat versus meats).

Concentrations of some trace elements in hair of patients with prostate cancer

S. A. K. Saleh, H. M. Adly. Um AlQura University, Faculty of Medicine, Biochemistry Department, Makkah, Saudi Arabia

Backgrounds: Deficiency or excess of trace elements can induce metabolic disorders and cellular growth disturbance, even mutation and tumorigenesis. Many authors observed direct association between micronutrient deficiencies and the cancer mortalities. Prostate cancer ranked the sixth most common cancer among males in Saudi Arabia and there are few studies of the association between trace element levels and prostate cancer in Saudi Arabia. Objective: This study aimed to explore the association between concentration of selected hair trace elements including selenium, zinc, copper, manganese and iron as long-term biological markers with prostate cancer in Saudi Arabia. Patients and Methods: The study included 88 patients with prostate cancer, 64 benign prostate hyperplasia (BPH) patients and 52 healthy male subjects of matched age. Full history and clinical data were recorded for all subjects. Prostate cancer patients were underwent digital rectal examination (DRE), trans-rectal ultrasonography (TRUS) guided biopsy of the prostate, computed tomography scan of the pelvis, bone scan and histopathological examination, accordingly prostate cancer stages and metastatic disease were confirmed. Prostate cancer patients were classified into localized (n = 46) and metastatic prostate cancer (n = 12). Hair samples collected from the nape section of all subjects and hair trace elements Se, Zn, Cu, Mn and Fe levels were analysed by ICP-MS (Perkin Elmer 7300D). Odd Ratio (OR) of trace elements levels in hair were adjusted for family history and smoking. Results: Mean Se and Zn levels in hair of the prostate cancer group were significantly lower as compared to BPH and healthy groups (p<0.05) whereas mean levels of hair Cu, Mn and Fe were significantly higher in prostate cancer than BPH and healthy groups (p<0.05). Mean hair levels of Se and Zn were significantly differentiated between localized and metastatic prostate cancer (p<0.05) whereas mean hair levels of Cu, Mn, Ni and Fe failed to differentiate these groups (p>0.05). Conclusion: Prostate cancer may be associated with trace element metabolic disorders. Low levels of Se and Zn and high levels of Cu, Mn and Fe appear to be associated with the risk of prostate cancer in Saudi Arabia. Additional prospective studies are needed to confirm the inverse association between Se and Zn levels and prostate cancer.
a fast and sensitive high performance liquid chromatography (HPLC) method to measure the major forms of vitamin A (retinol) and vitamin E (α-tocopherol and γ-tocopherol) in human plasma. **Methods:** Two hundred μL of plasma samples and 300 μL internal standard solution (5.0 mg/L of α-tocopherol acetate in ethanol) were mixed followed by addition of 1.0 mL of hexane. After vortex and centrifugation, the supernatant was removed, dried, and reconstituted in 200 μL of freshly made 3:1 mix of methanol: diethyl ether of which 25 μL was injected for HPLC analysis. Chromatographic separation was achieved using a C18 column (4.6x75 mm, 3.5 μm) with isocratic methanol elution at 1.8 mL/min. The chromatographic time between injections was 4.0 min. Retinol was detected by UV, whereas tocopherols were monitored by fluorescence. The six-point calibration was traceable to a NIST standard material (SRM 968e). Forty left over patient specimens were used to compare this method with an independent HPLC-UV method. Plasma samples collected from 51 healthy donors were analyzed to establish the reference interval. **Results:** Intra-assay and total coefficient of variation were <6.0% at three levels tested. No interference was found from lipemic, hemolytic, icteric and uremic samples. Other performance validation data are listed in the Table. Agreement results were obtained for retinol and α-tocopherol comparing to the HPLC-UV method. Discrepancy in the γ-tocopherol measurement was likely due to differences in the calibration methods as the independent HPLC-UV method used α-tocopherol calibration curve to quantify γ-tocopherol.

**Conclusion:** This HPLC method offers rapid and sensitive quantification of vitamins A and E in human plasma.

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**Table. Assay Characteristics**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity Range (ng/mL)</th>
<th>Accuracy of Obtained Concentration (%)</th>
<th>Reference Intervals (ng/mL)</th>
<th>Method Comparison</th>
<th>Standard Error of Estimate (ng/mL)</th>
<th>Bias (%)</th>
<th>Mean Recovery of Added Substance (%)</th>
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<tr>
<td>Retinol</td>
<td>2.5-50</td>
<td>91.5-105.4</td>
<td>2.91-8.20</td>
<td>0.80</td>
<td>0.06</td>
<td>0.09</td>
<td>93.2</td>
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<tr>
<td>α-tocopherol</td>
<td>10-200</td>
<td>97.5-102.0</td>
<td>5.0-21.9</td>
<td>1.13</td>
<td>0.18</td>
<td>0.04</td>
<td>99.6</td>
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<tr>
<td>γ-tocopherol</td>
<td>10-200</td>
<td>97.5-102.0</td>
<td>5.0-21.9</td>
<td>1.13</td>
<td>0.18</td>
<td>0.04</td>
<td>99.6</td>
</tr>
</tbody>
</table>

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**B-234**

**Dynamics of 3-epi-25-hydroxyvitamin D3 in Premature Infants During Neonatal Intensive Care Unit Hospitalization**

A. Anderson-Berry, G. Iong, E. Lyden, M. Kaufmann, C. Hanson. 1University of Nebraska Medical Center, Omaha, NE, 2Queen’ s University, Kingston, ON, Canada

Objective: Evaluate concentrations of 25(OH)D3 and 3-epi-25(OH)D3 in premature infants over time. Relevance: Availability of a valid biomarker to assess vitamin D status of infants is of great clinical importance. The interpretation of 25(OH)D3 in infants is complicated by the presence of a 3-epi-25(OH)D3 isomer that may falsely elevate the 25(OH)D3 concentration, while functional significance of this metabolite remains unclear.

Methods: 32 infants <32 weeks gestation were randomized to receive 400 or 800 IU/day of vitamin D3 orally. Serum samples were obtained monthly. Vitamin D metabolites were analyzed in triplicate using LC-MS/MS. Comparisons of categorical data was done using Fisher’s exact test; continuous data was compared using the Wilcoxon Rank Sum test. Spearman correlation coefficients were used to assess the correlation of vitamin D metabolites. Measurements over time were fit with linear mixed effect models. P<0.05 was considered statistically significant.

Results Mean gestational age at birth was 30.5 weeks; mean birth weight was 1405 grams. Mean serum 25(OH)D3 concentrations in cord blood were 17.3 ng/mL; mean levels at 8 weeks were 18.6 ng/mL. The percent of the total 25(OH)D3 concentration that was 3-epi-25(OH)D3 increased significantly (p<0.0001 for cord blood vs. 8 weeks, figure 1). 25(OH)D3 and 3-epi-25(OH)D3 concentrations were highly correlated at each time point (r=0.86, 0.61, 0.73, and p<0.0001 for cord, 4 week, and 8 weeks, respectively).

Conclusion 3-epi-25(OH)D3 concentrations were low in cord blood, but by 4 weeks gestational age, accounted for a significant proportion of the 25(OH)D3 level in this population. The high degree of correlation between total 25(OH)D3 levels and 3-epi-25(OH)D3 is consistent with 25(OH)D3 serving as the primary substrate for C3-epimerization. Vitamin D supplementation was effective in raising 25(OH)D3 levels, however significant increases in 3-epi-25(OH)D3 also occurred.

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**B-235**

**Determination of 25-(OH)-vitamin D3 and D2 in postmenopausal women and results comparison between immunochemical and chromatographic methods**

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Background: Objective of this study was to evaluate and validate HPLC method for determination of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 and to measure 160 patient samples and compare the results obtained by immunochemical and chromatographic measurements.

Methods: For determination of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 we used high performance liquid chromatography (HPLC) with UV detection (Agilent 1200). The samples were measured by kit (Recipe, Germany). The separation was accomplished at 40 °C, samples were detected at 264 nm. Chemiluminescent immunoanalysis was performed on Abbott Architect i4000SR analyzer (Abbott Laboratories, Germany). We measured 160 patient samples from postmenopausal women with osteoporosis and postmenopausal women without osteoporosis. For statistical evaluation we used GraphPad Prism 6.0.

Results: A linear relationship between serum concentration and peak area was obtained for both substances with correlation coefficient r=0.9989 for 25-(OH)-vitamin D3 and r²=0.9986 for 25-(OH)-vitamin D2. The limit of detection for 25-(OH)-vitamin D3 was 4.9 nmol/L and for 25-(OH)-vitamin D2 13.8 nmol/L. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis. Intraday CV for 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 were 5.7% and 2.8%. Intraday CV were 3.7% and 6.4%. Within-day accuracy expressed by the calculated bias between observed and theoretical concentrations for 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 were 4.6% and 6.7%. Mean recoveries of the corresponding compounds were 94.5% and 104%. No interference has been found between 25-(OH)-vitamin D3 and 25-(OH)-vitamin D3 or internal standard. We also compared the levels of 25-(OH)-vitamin D3 measured by HPLC with the levels of 25-(OH)-vitamin D measured by immunochemical method. Furthermore, we compared the sum of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 with 25-(OH)-vitamin D. The first data were tested by Mann Whitney test, the second part by unpaired t test. The data showed significantly differences, p = 0.0061 for comparison of 25-(OH)-vit D3 with 25-(OH)-vit D, and p = 0.0001 for the sum of 25-(OH)-vit D3 and 25-(OH)-vit D with 25-(OH)-vit D.

Conclusion: We assumed that the data measured by immunochemical method will be higher than the data measured by HPLC due to the large number of cross-reactions, but the results (expressed as median ± SEM) were contrary: 75.3 ± 38.8 nmol/L measured by HPLC vs. 64.7 ± 23.3 nmol/L measured by immunochemical method. Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic)
Comparison of the Roche Cobas Electrochemiluminescent Vitamin D Assay to the DiaSorin Chemiluminescent Method

R. Rosecrans, J. Greene. NorthShore University HealthSystem, Highland Park, IL

Background: Low vitamin D has been implicated in cancer, cardiovascular disease, diabetes, multiple sclerosis, autoimmune diseases, even autism, and headaches resulting in an exponential increase in vitamin D testing. Clinical diagnostic manufacturers have responded in meeting the vitamin D testing demand. Immunoassay manufacturers of vitamin D assays claim their methods correlate to LC/MS/MS, the reference method, but the literature has been varied. This study compares the Roche Cobas (Roche Diagnostics, Indianapolis, IN) electrochemiluminescence binding assay to the DiaSorin (Stillwater, MN) chemiluminescent assay for vitamin D.

Methods: The primary method used by the laboratory is the DiaSorin Total Vitamin D assay. 200 patient samples were chosen for the comparison study. To insure an adequate concentration distribution, patient samples were grouped as follows: less than 10.0 ng/mL, 10.1 to 20.0 ng/mL, 20.1 to 40.0 ng/mL, 40.1 to 60.0 ng/mL, and greater than 60.0 ng/mL. After determination on the DiaSorin Liaison, the samples were frozen and held until transport to a laboratory performing the Roche Cobas assay.

Results: Linear regression analysis between the Roche Cobas and the DiaSorin Liaison demonstrated a correlation coefficient of 0.7272. Bland-Altman plots demonstrate value differences ranging from -0.3044 mg/mL to +0.54 mg/mL. Patient subgroups were divided and analyzed separately. One patient group (n = 91) had values between 5 to 20 mg/mL as determined by the DiaSorin method. Linear regression analysis of this subgroup demonstrates a correlation coefficient of 0.6749. The second population subgroup (n = 172) had DiaSorin vitamin D results between 20.1 to 40.0 ng/mL and a correlation coefficient of 0.1742 when compared to the Roche Cobas assay. Roche Cobas had an overall positive bias compared to the DiaSorin that resulted in 48%, 15.8%, and 14.8% fewer patients being classified as deficient (<10 ng/mL), insufficient (10.1-31 ng/mL), and sufficient (>31 ng/mL), respectively. The Bland-Altman analysis demonstrated slight concentration-dependent bias. Overall, Passing-Bablok fit showed the in-house LC-MS/MS method was statistically equivalent to Chromsystems (p value > 0.05) with a high to very high correlation coefficient (r = 0.876 to 0.986). Table 1. The ability to properly classify patients according to their vitamin D status was very satisfactory for the tested method which the Kappa values were 0.864, 1.00 and 0.944 for total 25OH-D, 25OH-D3, and 25OH-D4 (concordance >90%), respectively.

Conclusion: The in-house LC-MS/MS method for total 25OH-D, 25OH-D3, and 25OH-D4 determination correlated very well with the NIST traceable method. Strength of agreement for classifying patients into the low or optimal vitamin D levels with the reference assay was very good. The observed bias had little impact on clinical decision therefore is clinical acceptable. We conclude that our LC-MS/MS method met the minimum requirements for the assessment of vitamin D status in clinical laboratories.

Table 1: Passing-Bablok regression analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>Intercept</th>
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<th>Slope</th>
<th>50% CI</th>
<th>p-value</th>
<th>r</th>
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<tr>
<td>25OH-D</td>
<td>0</td>
<td>21.5</td>
<td>3.7</td>
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<tr>
<td>1,25-D</td>
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<td>29.1</td>
<td>4.2</td>
<td>0.002</td>
<td>0.058</td>
<td>0.663</td>
<td>0.643</td>
<td>0.002</td>
<td>0.663</td>
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</table>

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Evaluation of a Random Access Total 25-Hydroxy Vitamin D (THVD) Immunoassay (IA): Patient Correlation with HPLC-Mass Spectrometry (MS)

N. Yadak1, S. Freeman2, C. Hoang3, C. Finch Cruz4, E. S. Pearlman5.

1University of Tennessee Health Sciences Center, Memphis, TN; 2Veterans Administration Medical Center, Memphis, TN; 3Veterans Affairs Medical Center, Memphis, TN

Background: The VAMC evaluated a THVD assay to be employed on our Vitros-5600 platform [OCD, Raritan, NJ]. Specimen results with compared to HPLC-mass spectrometry (MS) taken to be the reference method.

Methods: Specimens (n=42) received frozen from a reference lab [(RL)/ARUP; Salt Lake City, UT] had been assayed for THVD using MS. These were thawed shortly before IA and run in random order according to manufacturer’s directions. Two of the specimens had IA values below the analytical measurement range and were excluded from the regression analysis. There were 13 samples with adequate residual volume to be returned as blinded liquid samples and re-assayed with MS. In a separate study 40 in-house specimens were run twice using IA with the specimens being stored at 4 degrees overnight between assays. Regression analysis used Table Curve-2D software (Systat; San Jose, CA).

Results: The relationship between IA (Y) and MS (X) data was fit reasonably well by the linear equation [with 95% CIs]:

Y = 1.015 [0.888, 1.15] • X + 1.25 [-0.59, 3.00] (n=40, r-sq = 0.856).

Using a THVD concentration of 30 mcg/L as the threshold for optimality there were six specimens with MS values < 30 mcg/L that were > 30 mcg/L by IA and 2 specimens with the reverse situation. The median (range) within-pair precision for IA (n=40) was 2.56% (0.11-8.32%) with the CV being -10% in 39/40 instances. A plot of CV (Y) vs. Mean (X) suggests a non-linear relationship with a CV that decreases with increasing mean concentration but the 95% CI on the slope includes zero. The median (range) within pair precision (n=13) using MS was 10.5% (2.30-9.4%) with 4 specimens having a CV >20%. All MS specimens with initial THVD i concentrations of >30 mcg/L (n=8) however remained optimal and likewise for those initially with sub-optimal THVD.

Conclusions: The overall performance of the IA from OCD appears satisfactory but there may be some increase in the number of patients with suboptimal concentrations with IA compared to MS. IA reproducibility after 24 hours of refrigeration was very good. Although the number of specimens retested by MS was small there is the suggestion that a freeze thaw cycle and transportation time may result in significant imprecision although clinical reclassification was not observed.
High prevalence of Vitamin D Deficiency in Korean pregnant women and association between maternal 25-hydroxyvitamin D level in pregnancy and neonatal outcomes

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Background: There is growing concern about functional impacts of maternal vitamin D status on multiple adverse health outcomes in mothers and on their offspring and low maternal levels of 25-hydroxyvitamin D [25(OH)D] has been suggested to be associated with some adverse obstetrical and neonatal outcomes. However, there were no reliable data based estimation of vitamin D status using LC-MS/MS in Korean pregnant women. Objective: The aim of this study was to carry out the first population-based survey on vitamin D status in Korean pregnant women to assess vitamin D status during pregnancy and the effect of vitamin D deficiency on pregnancy outcomes; premature rupture of membrane, preterm birth, and child born small for gestational age. Method: Korean pregnant women (n=220) were recruited prospectively and tested for 25(OH)D levels in serum using liquid chromatography-tandem mass spectrometry with assessment of maternal characteristics. Their 25(OH)D levels were compared with those of 500 healthy nonpregnant women. We analyzed tandem mass spectrometry with assessment of maternal characteristics. Their 25(OH)D levels were compared with those of 500 healthy nonpregnant women. We analyzed tandem mass spectrometry with assessment of maternal characteristics. The median concentrations of 25(OH)D in Korean pregnant women (n=220) and healthy nonpregnant women were 77.3% (170/220) and 79.2% (396/500), and the prevalence of severe vitamin D deficiency [25(OH)D < 10 ng/mL] were 28.6% (63/220) and 7.2% (36/500), respectively. The prevalence of vitamin D deficiency was higher in winter (100%) than in summer (45.5%) in Korean pregnant women. The 1st trimester had a higher risk of vitamin D deficiency than the 3rd trimester (adjusted OR 4.3; 95% CI 1.2-15.2; P = 0.05). No associations were observed between vitamin D deficiency and pregnancy or birth outcomes including premature rupture of membrane, preterm birth, and child born small for gestational age. Conclusions: The prevalence of vitamin D deficiency was high in pregnant women in Korea and showed the highest during the 1st trimester of pregnancy. Although there was no association between vitamin D deficiency and pregnancy outcome, further research about the long term consequences of vitamin D deficiency during pregnancy on the mother and the offspring is warranted.

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NUTRITION/TRACE METALS/VITAMINS

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Performance Characteristics of the ARCHITECT Active-B12 (Holotranscobalamin) Assay: A Marker of Vitamin-B12 Deficiency

S. D. Merrigan, W. E. Owen, J. A. Straseski. ARUP Laboratories, Salt Lake City, UT

Background Vitamin B12 (cobalamin) is a necessary cofactor in methionine and succinylic-CoA metabolism. Some studies estimate the prevalence of deficiency may be as high as 30% in the elderly population. Cobalamin deficiency is a serious health risk due to its role in carbon metabolism, cell division, DNA synthesis and the clinically important outcomes of anemia and progressive and irreversible neurologic dysfunction. Ten to thirty percent of circulating cobalamin is complexed with transcobalamin and is called holotranscobalamin (holoTC). HoloTC can readily enter cells and is therefore considered the bioactive form. The objective of our study was to evaluate the analytical performance of the ARCHITECT 2000SR Active-B12 (Holotranscobalamin) assay and compare results to manual and automated immunoassays.

Methods Manufacturer-specified limits of blank (LoB), detection (LoD), and quantitation (LoQ), imprecision, interference and linearity were evaluated for the ARCHITECT Active-B12 (Holotranscobalamin) assay (Abbott Diagnostics, Abbott Park, IL) per CLSI guidelines. Residual de-identified serum samples were used to compare results from the ARCHITECT HoloTC assay with the results from the automated Abbott AxsYM Active-B12 (Holotranscobalamin) assay (Abbott Diagnostics) and the manual Active-B12 (Holotranscobalamin) Enzyme Immunoassay (EIA) (Axis-Shield Diagnostics, Dundee, Scotland, United Kingdom).

Results Manufacturer’s claims of <0.4, <1.9 and <5.0 pmol/L for LoB, LoD, and LoQ, respectively, were verified for the ARCHITECT HoloTC assay. Total within-assay imprecision was 5.7% at a mean concentration of 16.2 pmol/L (SD 0.9 pmol/L), and 4.9% at a mean concentration of 46.7 pmol/L (SD 2.3 pmol/L), verifying the manufacturer’s imprecision claims. Interference studies demonstrated <10% deviation in recovery for hemolysis, icterus, and lipemia up to concentrations of 200 mg/dL, 20 mg/dL, and 850 mg/dL, respectively. The ARCHITECT HoloTC assay was linear up to the highest concentration measured (113.4 pmol/L). The largest mean deviation from the calculated recovery was 8.5% at an expected holoTC concentration of 26.2 pmol/L. Method comparison of the ARCHITECT HoloTC assay to the AxsYM HoloTC assay for samples with results within the AMR gave the following Deming regression statistics with 95% confidence intervals: (ARCHITECTHoloTC) = 0.941 ± 0.062(AxsYM HoloTC) + 1.2 ± 2.5 pmol/L, Syx = 6.4, r = 0.947 (n=98). Average bias between the methods was 0.9 pmol/L (-3%). Method comparison of the ARCHITECT HoloTC assay to the Active-B12 EIA gave the following Deming regression statistics with 95% confidence intervals: (ARCHITECTHoloTC) = 1.105 ± 0.046(AxesYM Active-B12) – 6.8 ± 2.7 pmol/L, Syx = 11.0, r = 0.950 (n=221). Average bias between methods was -1.7 pmol/L (-3%). A medical decision point analysis at 35.0 pmol/L was calculated using Deming statistics. The AxsYM HoloTC assay agreed within the 95% confidence intervals of the ARCHITECT assay (33.7 – 35.7 pmol/L), while the Active-B12 EIA comparison was slightly below (31.7 – 34.2 pmol/L).

Conclusions Panel of blood biomarkers may be useful in predicting key clinical outcomes such as LBM loss over immobilization (e.g. hospitalization). Validation of these markers in large clinical studies is needed. Sponsored by Abbott Laboratories.
An additional comparison to a manual Active-B12 EIA method performed similarly, with minor exceptions. This study determined that the ARCHITECT HoloTC assay is suitable for routine clinical use.

**B-245**

**Achieving 25(Oh)vitamin D and 25(Oh)vitamin D2 Equimolarity for the Dimension® EXL™ Vitamin D Total Assay**

E. Garcia, W. Bedzyk, J. Li, S. Manoj, T. Q. Wei. Siemens Healthcare Diagnostics Inc., Newark, DE

**Background:** The Dimension EXL Vitamin D Total (VitD) assay is a homogenous, competitive immunoassay based on LOCI® technology. The VitD assay utilizes 4 reagents: a releasing reagent, two latex bead reagents and a biotinylated monoclonal antibody reagent. The releasing reagent releases 25(OH)vitamin D$_2$ and 25(OH)vitamin D$_3$ from the endogenous vitamin D binding proteins (DBP). The released 25(OH)vitamin D$_2$ and 25(OH)vitamin D$_3$ are then bound by the biotinylated assay antibody to produce the assay signal. The assay is intended for the equimolar determination of 25(OH)vitamin D$_2$ and 25(OH)vitamin D$_3$.

**Methods:** Recovery of 25(OH)vitamin D$_2$ or 25(OH)vitamin D$_3$ was assessed using the 25(OH)vitamin D$_2$ or 25(OH)vitamin D$_3$ spiked human serum samples. Available anti-25(OH)vitamin D monoclonal antibodies were screened for anti-25(OH)vitamin D$_2$ specific binding using a prototype LOCI VitD assay. One anti-25(OH)vitamin D$_2$ antibody was selected and added to the biotinylated antibody reagent to bind the excess 25(OH)vitamin D$_2$, which led to 25(OH)vitamin D$_2$ /25(OH)vitamin D$_3$ equimolarity.

**Results:** A dose response curve showed the recovery ratio of 25(OH)vitamin D$_2$ /25(OH)vitamin D$_3$ changed from 118% to 84% with the increase of anti-25(OH)vitamin D$_3$ antibody from 0 to 80 μg/mL. Adding 20 μg/mL of the antibody in the assay reagent achieved 100% recovery ratio or 25(OH)vitamin D$_2$ /25(OH)vitamin D$_3$. 

**Conclusion:** By achieving 25(OH)vitamin D$_2$, 25(OH)vitamin D$_3$ equimolarity and employing highly sensitive LOCI technology, the fully automated Dimension EXL Vitamin D Total assay provides accurate and precise total 25(OH)vitamin D measurement on the Dimension EXL system.

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


**B-246**

**Development of a Vitamin D Total Assay® with LOCI® Technology on the Dimension® EXL™ System**


**Background:** The Siemens Dimension EXL System incorporates multiple detection technologies, including LOCI technology, which enables high sensitivity immunoassay formats. Siemens is currently developing a Vitamin D Total assay utilizing LOCI technology on the Dimension EXL System.

**Methods:** The Dimension EXL Vitamin D Total assay (VitD) is a homogenous competitive chemiluminescent immunoassay based on LOCI technology. It measures the total 25(OH)vitamin D concentration (comprised of 25(OH)vitamin D$_2$ and 25(OH)vitamin D$_3$) in both serum and plasma. The VitD LOCI components include a releasing reagent, two synthetic bead reagents and a biotinylated monoclonal antibody. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a 25(OH)vitamin D$_2$ analog and contains chemiluminescent dye. The sample is incubated with the releasing reagent to release 25(OH)vitamin D$_2$ molecules from the vitamin D binding proteins. The reaction mixture is then incubated with biotinylated antibody to form a 25(OH)vitamin D/biotinylated antibody complex. Chemibeads are added to remove the excess free biotinylated antibody, and then Sensibeads are added to bind to the biotinylated antibody. Aggregates of the Chemibead-analog/antibody-biotin/streptavidin-Sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH)vitamin D in the sample.

**Results:** The method requires 8 μL of serum or plasma and is linear from 4 to 150 ng/mL. Time to first result is 32 minutes with a stable calibration for 7 days.

**B-247**

**Development of Candidate Standard Reference Material® 3949 Folate Vitamers in Frozen Human Serum**

J. E. Camara, M. S. Lowenthal, J. S. Pritchett, K. W. Phinney, L. C. Sander. NIST, Gaithersburg, MD

The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials (SRMs) for the analysis of nutrient levels in clinical matrices. NIST currently offers SRM 1955 Homocysteine and Folate in Human Serum, which possesses certified values for homocysteine and 5-methyltetrahydrofolate (5-mTHF) and reference values for folic acid, also known as pteroyl-glutamic acid (PGA), over three concentration levels of material. SRM 1955 Level 1 required dilution and SRM 1955 Level 3 required spiking of a serum pool to achieve the target concentrations. Once out of stock, this SRM will be replaced with a new candidate material, SRM 3949 Folate Vitamers in Frozen Human Serum. Input from The National Institutes of Health Office of Dietary Supplements (NIH-ODS) and the Centers for Disease Control and Prevention (CDC) indicates interest in an updated material with folate levels reflecting those currently observed in the population. This new SRM will have three concentration levels with low, medium, and high certified values for both 5-mTHF and PGA. NIST also intends to assign reference values for the additional minor folate metabolites tetrahydrofolate (THF), 5-formyltetrahydrofolate (5-THF), 5,10-methylenetetrahydrofolate (5,10-methenylTHF), and the oxidation product of methyl folinate (MeFox). The goal levels for 5-THF and PGA, respectively, are: Level 1, 10 nmol/L and 1 nmol/L; Level 2, 50 nmol/L and 10 nmol/L; Level 3, 30 nmol/L and 5 nmol/L. In addition, Level 3 has goal levels of 5 nmol/L, 5 nmol/L, 5 nmol/L, and 3 nmol/L for THF, 5-THF, 5,10-methenylTHF, and MeFox, respectively.

To produce SRM 3949, pilot sera were collected from 15 individual donors, five of which were given a 400 μg folic acid supplement one hour prior to blood draw in an attempt to increase serum levels of 5-mTHF and PGA for the high level material without the requirement for additional spiking. To stabilize the folates, 0.5 % (w/v) ascorbic acid was added as soon as possible after collection of serum. These pilot sera were screened for five folates plus the oxidation product, MeFox, at the CDC by ID-LC-MS/MS. Screening results ranged from 5 nmol/L-72 nmol/L for 5-THF, 0.4 nmol/L-32 nmol/L for PGA, 0.25 nmol/L-2.2 nmol/L for THF, and 0.13 nmol/L-3.2 nmol/L for MeFox. Both 5-THF and 5,10-methenylTHF were below the limits of detection for all sera. Four pilot sera from donors administered a folic acid supplement displayed significantly elevated levels of both 5-mTHF and PGA. Based on these results, a blending protocol was specified to obtain the desired folate concentrations in each of the three SRM 3949 concentration levels. The endogenous levels of 5-mTHF and PGA in all three concentration levels and enhanced folate stability via ascorbic acid addition are improvements over the original SRM 1955 that should better serve the end users.

The candidate material has been blended and packaged and will undergo additional analyses by ID-LC-MS/MS at both NIST and the CDC. NIST is also investigating updates to its current ID-LC-MS/MS method for folates in serum based on the current CDC method, which may be applied to the certification measurements of SRM 3949.
Wednesday, July 30, 9:30 am – 5:00 pm

B-248
The Frequency of Vitamin B12 Deficiency in Metformin-treated Brazilian Type 2 Diabetes Patients.


Background: Vitamin B12 is an essential micronutrient required for optimal hematopoietic, neurocognitive and cardiovascular function. Metformin is a biguanide recommended as initial medical therapy for type 2 diabetes mellitus (T2DM). Despite the known effectiveness, there are disadvantages in its use. Studies indicate a prevalence of 14% to 30% of vitamin B12 deficiency among patients undergoing long-term treatment with metformin, with a still controversial mechanism. The objective of this study was to evaluate the frequency of vitamin B12 deficiency and the factors associated with serum vitamin B12 levels in metformin-treated Brazilian type 2 diabetes patients.

Methods: Cross-sectional study that included 231 T2DM patients in metformin therapy. All the patients were followed at the Endocrinology Division of the University Hospital. The serum B12 levels were measured by chemiluminescence method (Access, Beckman Coulter, CA, USA). Vitamin B12 deficiency was defined by a serum level below 180 pg/mL. SPSS 13.0 was used for statistical analysis. The Mann Whitney test was used to compare numerical variables between groups, the McNemar test to assess the association between binary variables. p value <0.05 was considered to be statistically significant.

Results: Median age was 61 (34 to 79) years and 72% were women. The median time of T2DM was 12 (3 to 41) years, duration of metformin use was 8 (3 to 30) years and dose of metformin was 1,700 (500 to 2,550) mg/day. The vitamin B12 mean levels were 272 (68 to 1,000) pg/mL. The frequency of cobalamin deficiency was 26.8%. The patients with vitamin B12 deficiency had longer disease duration (14 vs 10 years; p=0.042) and longer metformin use (10 vs 8 years; p=0.016). Vitamin B12 deficient patients were significantly lower in patients that were using H2 antagonists or proton pump inhibitors (210 vs 292 pg/mL; p=0.002). After multiple regression analysis, only duration of metformin treatment and the use of H2 antagonists / proton pump inhibitors were significantly correlated to cobalamin levels.

Conclusion: Our study confirmed that the frequency of vitamin B12 deficiency is high in metformin-treated T2DM patients. The patients with vitamin B12 deficiency were using metformin for a longer time, suggesting a cumulative effect of the drug. The use of H2 antagonists or proton pump inhibitors negatively influenced the serum levels of vitamin B12, demonstrating the role of acid gastric reduction as a predisposing factor to vitamin B12 deficiency. Finally, as vitamin B12 deficiency is a cause of peripheral neuropathy, it should be considered in the differential diagnosis of diabetic neuropathy.

B-250
An Improved Cleanup Strategy for Patient Samples using Anion Exchange Solid Phase Extraction for the Analysis of Vitamin B6 Status

M. Borasaki1, S. Baek2, N. Bhavsar3, M. Pikulski4. 1Clinical Pathology Laboratories, Austin, TX, 2Biotage, Charlotte, NC

Background: Low levels of vitamin B6 are implicated in health problems involving the nerves, skin, mucous membranes, and circulatory system. In children, vitamin B6 deficiency is related to cases of anemia and seizures. Vitamin B6 dependent seizure disorders are an important and treatable cause of childhood epilepsy. Pyridoxal 5’-phosphate (PLP), the primary biologically active form of vitamin B6, is the preferred method for assessing vitamin B6 status. Most of the published procedures for PLP involve extraction via protein precipitation from plasma followed by derivatization to enhance the fluorescence signal. In this work, we present a more selective sample extraction by using anion exchange solid phase extraction (SPE). This procedure is also followed up with derivatization. Our objectives were to automate the procedure by using SPE, decrease the noise and increase the resolution in the chromatography, improve the HPLC column lifetime and create a more robust method with cleaner sample extracts.

Method: To 200µL of plasma, 30µL semicarbazide/glycine derivatizing reagent was added. The samples were then vortexed and incubated. The samples were then processed using strong anion exchange SPE cartridges (EVOULATE AX from Biotage). The eluent was then evaporated and reconstituted in HPLC grade water. After vortexing, 60µL of the sample was then injected onto a HPLC-FLD equipped with a pre-column (KmndKatcher, Phenomenex) and a 4.6x100mm, 3µ, Gemini-NX C18 HPLC column (Phenomenex). HPLC mobile phase A was 10mM sodium phosphate dibasic in 0.3% acetic acid and mobile phase B was acetonitrile/methanol (70:30) in a 5 minute gradient (1mL/min). The excitation and emission wavelengths were optimized at 370 and 470nm, respectively.

Results: The improvements in our method using the SPE cleanup included increased column lifetime (3X), improved resolution and chromatographic robustness and improvements in the run failure rate. The recovery was increased from 73% to 91%. Three levels of plasma quality controls (QC’s) were tested at 33, 92, and 360mM/L. The first two QC’s were commercially available (Chromsystems) and the third was prepared in-house from pooled patient samples. The coefficient of variation percent (CV%) for intra-assay precision for the QC’s were 5.1%, 4.6%, and 6.9% and inter-assay precision were 10.3%, 5.3%, and 7.3%. The linear regression analysis data for six calibration curve points spanning 2,400mM/L, yielded an R2=0.9994. The lower limit of quantitation (LLOQ) is 2mM/L. A method comparison was performed with an outside laboratory utilizing 50 patient samples. The Deming regression analysis resulted in correlation R=0.96 for PLP using HPLC-FLD.

Conclusion: An improved method for the analysis of pyridoxal 5’-phosphate has been developed and has proven to be accurate, precise and robust.
Reference intervals for intestinal disaccharidase activity determined from a non-reference population

S. A. Hackenmueller, D. G. Grenache. University of Utah, Salt Lake City, UT

Background: Dietary disaccharides are important exogenous sources of glucose. Because the small intestine is normally impermeable to disaccharides, the activities of intestinal disaccharidases are required for hydrolysis into component monosaccharides that are subsequently absorbed. Decreased or absent activities of one or more disaccharidasises can result in carbohydrate malabsorption. The measurement of disaccharidase activities in small intestine mucosa is considered the gold standard test for diagnosis of disaccharidase deficiency. Due to the inability to obtain intestinal biopsies from a healthy reference population, laboratories that perform disaccharidase testing have adopted historical cutoff activities that are used to identify a disaccharidase deficiency (lactase, <15; maltase, <100; palatinase, <5; and sucrase, <25 U/g protein). The objectives of this study were to validate these historical activity cutoffs using the Hoffmann method for reference interval determination and to evaluate disaccharidase activities and demographics of individuals for whom intestinal disaccharidase testing was performed.

Methods: 14,827 samples for which all four disaccharidase test results were available were extracted from the laboratory information system. For cutoff validation, results of 0 U/g protein were excluded. Enzyme activities were log transformed and the Hoffmann method was used to calculate a reference interval. This method involved determining the cumulative frequency distribution for each enzyme and performing linear regression over the linear portion of the distribution. The reference interval (RI) was calculated as RIlower=2.5(m)+b and RIupper=97.5m+b (m=slope; b=intercept). The frequencies and patient demographics of all possible disaccharidase activity phenotypes were determined from the entire population.

Results: The reference intervals for each enzyme were calculated to be 5.55 for lactase, 105-380 for maltase, 9.92 for palatinase, and 26-110 U/g protein for sucrase. The ratios of historical cutoffs to the calculated lower reference limits were 0.95, 0.56 and 0.96 for maltase, palatinase and sucrase, respectively, indicating the historical cutoffs were less than the lower reference limit for each enzyme. The ratio for lactase activity was 3, indicating the historical cutoff was within the calculated reference interval. Examination of the frequency distribution of the activities of each enzyme revealed that maltase, palatinase, and sucrase were unimodal while lactase showed a bimodal distribution. The intersection of the two lactase populations corresponded to a lactase activity of 10 U/g protein, which produced a ratio of 1.5 if taken to be the lower reference limit. The median patient age of the entire data set was 13 years (range, <1-88 years) and 45% were male. Using the historical cutoffs, 52% of samples had no enzyme deficiencies. Deficiencies of lactase, maltase, palatinase, and sucrase were present in 47, 11, 3, and 13% of the samples, respectively. 35% of samples were deficient only in lactase. 3% of samples were deficient in all four enzymes.

Conclusion: The historical cutoffs for maltase and sucrase were validated. To align with calculated reference intervals, the palatinase cutoff should increase to 9 U/g protein, and the lactase cutoff should decrease to 10 U/g protein. Disaccharidase testing is most commonly performed in patients <18 years. Lactase deficiency is the most frequently observed single-disaccharidase deficiency. Pandisaccharidase deficiency is rare.

B-253

Vitamin D Sufficiency Thresholds: Are Age-Specific Values Needed?

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Background: Vitamin D insufficiency has become a global health problem that has been associated with metabolic bone disease and a great variety of chronic illnesses. Thresholds for vitamin D sufficiency have been based mainly on total 25-hydroxyvitamin D (25OHD) concentrations at which serum parathyroid hormone (PTH) increases, but there is great controversy surrounding the precise level at which these changes occur. Current guidelines mainly suggest values of 20 or 30 ng/mL. Although vitamin D requirements are thought to vary with age, there are no differences on the reported thresholds. The aims of this study were to analyze the relationship between 25OHD and PTH in different age groups and to evaluate the need for specific reference values for different age groups.

Methods: This was a cross-sectional analysis of 23,276 paired serum PTH and 25OHD levels measured from January 2012 to December 2012 in a large Brazilian reference laboratory. Data on laboratory tests and demographic variables were available from a computerized database. Serum 25OHD was measured through Chemiluminescence, using the Architect® 25-OH Vitamin D Assay (Abbott, Illinois, USA). Serum PTH was measured through Chemiluminescence, using the Access® Intact PTH Assay (Beckman Coulter, California, USA).

Results: Laboratory tests were equally distributed throughout the different seasons of the year. Eighty-one percent of the studied population was female and the mean age was 54.7±18.3 years. Serum 25OHD ranged between 8 and 160 ng/mL, with a mean of 27.7±11.4. Serum PTH levels were inversely correlated with serum 25OHD. The 23,276 patients were split by their 25OHD values into 50 groups and the median PTH of each was calculated, as well as the rate of results exceeding the upper limit of PTH. Next, the data were broken down into five age classes (0 to 20, 20 to 40, 40 to 60, 60 to 80, and older than 80 years-old). The median PTH and the rate of high results were plotted against the mean 25OHD and the graphs demonstrated that the PTH concentrations were consistently higher in the oldest adults at different 25OHD levels. The median PTH associated with 25OHD concentration of 20 ng/mL in each age group was: 23 (0 to 20 years-old), 38 (20 to 40 years-old), 40 (40 to 60 years-old), 48 (60 to 80 years-old), and 57 ng/mL (over 80 years-old). When 25OHD concentration of 30 ng/mL was considered, the median PTH of each group was, respectively, 23, 32, 34, 43 and 46 pg/mL.

Conclusion: We concluded that the serum concentrations of 25OHD required to overcome hyperparathyroidism are different depending on the age and that the cut-
off values obtained in older adults shouldn't be applied to other groups. We assume that the development of specific reference values according to age will attenuate the overdiagnosis of vitamin D insufficiency and overtreatment of otherwise healthy young patients.

B-254

C-ing is Believing: Enhanced Specificity for Vitamin C using HPLC with Electrochemical Detection and Automatic Alternating Column Regeneration

Z. D. Clark¹, E. L. Frank². ¹ARUP Laboratories, Salt Lake City, UT, ²Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT

BACKGROUND: Vitamin C (L-ascorbic acid) is a water-soluble micronutrient that is essential for human health. Deficiency of vitamin C causes the fatal disease scurvy. Since the 1940’s, vitamin C has been measured spectrophotometrically after derivatization to form colored products. These methods are subject to lack of specificity, limited sensitivity, and interference from other compounds. Newer, more specific techniques utilize chromatographic separation. While a high-specificity mass spectrometer may be an appealing detector choice, we found electrochemical detection (ECD) provided adequate selectivity and sensitivity at a fraction of the cost.

OBJECTIVE: The goal of this study was to develop a high-throughput HPLC-ECD method for the measurement of vitamin C in plasma.

METHOD: Protein is precipitated from plasma using 10% meta-phosphoric acid. An internal standard, 3,4-dihydroxybenzylamine, is added and the solution is incubated with dithiothreitol in phosphate buffer to reduce dehydroascorbic to ascorbic acid. Following re-acidification, a 5 μL aliquot is injected onto an Agilent HPLC system. The analytes exiting the analytical column undergo an electrochemical reaction in a coulometric cell. The current generated is proportional to analyte concentration and is measured by the Coulochem®III detector. The new assay was developed and validated using a single pump and a single analytical column. The injection-to-injection time was 13 min. Subsequently, to increase the method throughput and shorten turnaround time, a dual LC pump system with a 2-position/10-port switching valve capable of performing automatic alternating column regeneration was validated and implemented. The injection-to-injection time was reduced 2-fold.

RESULTS:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ</td>
<td>1.9 μmol/L</td>
</tr>
<tr>
<td>AMR</td>
<td>5 - 5,000 μmol/L</td>
</tr>
<tr>
<td>Linearity</td>
<td>y=0.9775x+0.004; observed error 1.9%</td>
</tr>
<tr>
<td>Imprecision</td>
<td>Low Control: 23.62 μmol/L, 117.62 μmol/L</td>
</tr>
<tr>
<td></td>
<td>High Control: 5.2 %, 3.0 %</td>
</tr>
<tr>
<td></td>
<td>Within run CV: 3.6 %, 3.0 %</td>
</tr>
<tr>
<td></td>
<td>Total CV: 6.3 %, 3.7 %</td>
</tr>
<tr>
<td>Accuracy: Method Comparison</td>
<td>Discarded specimens (n=44): y=0.983x-8.93; Sx=7.42; R=0.9901</td>
</tr>
<tr>
<td></td>
<td>Fresh ref. interval specimens (n=41): y=0.834x-1.08; Sx=6.77; R=0.9167</td>
</tr>
<tr>
<td>Carryover</td>
<td>Not detected after injection of sample with 14,200 μmol/L of ascorbic acid (AA)</td>
</tr>
<tr>
<td>Analytical Specificity Interference</td>
<td>33 common drugs and endogenous compounds tested. Only isoascorbic acid (erythorbic acid), a non-endogenous stereoisomer of AA, co-eluted. Gross hemolysis (166 μmol/L) reduced measured AA concentration by 12%</td>
</tr>
</tbody>
</table>

CONCLUSION: We have successfully developed and validated an HPLC-ECD method for the measurement of vitamin C in plasma. Advantages of this method include higher analytical specificity and significantly simplified sample preparation compared to the previously used spectrophotometric method. Additionally, much shorter injection-to-injection time compared to HPLC methods utilizing a single LC column was achieved by employing an automatic alternating column regeneration system.