Wednesday, July 30, 2014

Poster Session: 9:30 AM - 5:00 PM

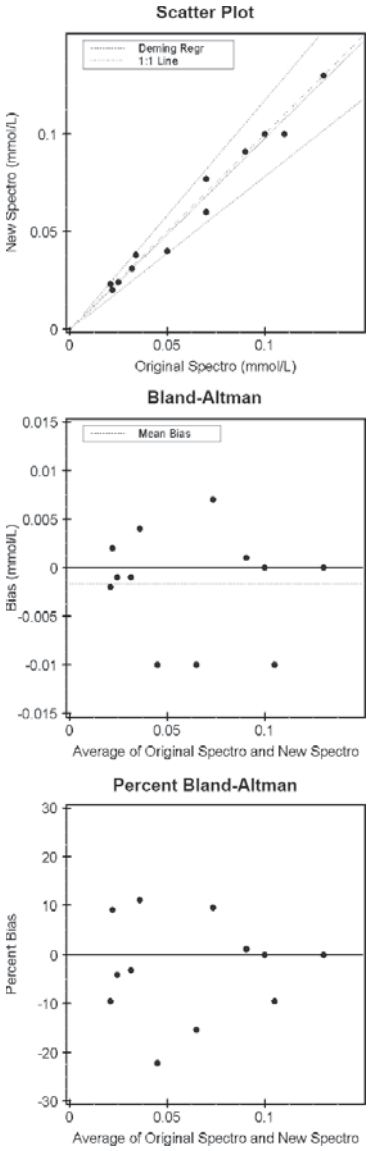
Electrolytes/Blood Gas/Metabolites

B-176

Validation of a spectrophotometric assay for the measurement of pyruvate in whole blood.

S. Albeiroti, J. M. El-Khoury, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Pyruvate plays a major role as an intermediate in carbohydrate and amino acid metabolism. Clinically, an elevated Lactate-to-Pyruvate ratio can be indicative of several diseases including disorders of mitochondrial metabolism and inborn errors of metabolism. In our laboratory, pyruvate is measured by a spectrophotometric assay. NADH used in the assay was prepared freshly before each test by adding Trizma® buffer to a 2 mg NADH bottle from Sigma Aldrich®. Inconsistency in absorbance values among different NADH bottles provided by the manufacturer has been a major challenge in our lab. **Objective:** To develop a pyruvate assay that requires shorter processing time and results in more consistent absorbance values. **Methods**: NADH stock (0.225 mg/ml) was prepared in 1.5 mmol/L Trizma® and stored at 4°C. Deproteinized blood sample was prepared by adding 2 mL whole blood to 4 mL cold 12% trichloroacetic acid. The mixture was mixed, incubated for 10 minutes at 4°C and centrifuged for 7 minutes at 2000 g. 2 mL of the supernatant was mixed with 1 mL 0.225 mg/ml NADH in a 1-cm cuvette. Using Beckman DU 800 spectrophotometer, absorbance was measured at 340nm. 50 µL of 1000 units/mL LDH was then added and absorbance was measured after 10 minutes of incubation. **Results:** NADH stock solution was stable at 4°C over a period of 60 days with an inter-assay precision of 1.8% for the pyruvate measurements in prepared QCs. QCs used were 0.07 mmol/L pyruvate in 5% BSA in saline. The assay had an analytical measurement range of 0.04-0.35 mmol/L with recoveries ranging from 89% to 105%. Average within-run precision was <1%. Pyruvate results of patient samples obtained by this assay (n=12) were comparable with results obtained by the original method (r=0.9891, slope=0.986, intercept=-0.0008, SEE=0.0058). **Conclusion:** The modified spectrophotometric assay for the measurement of pyruvic acid in whole blood is accurate and robust.



B-177

Validation of creatinine test using the standard (SRM967) as reference for two methodologies

A. L. Camilo, F. S. Fukuoka, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: The ADVIA Creatinine_2 is used for *in vitro* diagnostic determination of human serum, plasma (lithium, heparin) and urine creatinine activity on the ADVIA Biochemical Systems (Siemens Healthcare Diagnostics). These measurements are used in renal diseases diagnosis and treatment, and also in renal dialysis monitoring. **Objective:** This study aims to perform the validation of Creatinine_2 (CREA_2) ADVIA assay on ADVIA 2400 equipment using as reference the standard (SRM967) of the National Institute of Standards and Technology (NIST). Moreover, the study verifies if results obtained by the method had deviation no greater than the analytical specifications.

Methods: The Creatinine_2 (CREA_2) method is based on the reaction of picric acid with creatinine in an alkaline medium, as described in the original Jaffe´s procedure. The assay range is 0.1 - 25.0 mg/dL (8.84 - 2,210 µmol/L) to serum samples and 1.5 - 300 mg/dL (133 - 26,250 µmol/L) to urine samples.

Results: Data show the total imprecision of measurements using control material and prepared pools. The within-run assay obtained CV=0.74% to 2.6%, and the total assay obtained CV=2.55% to 5.01% on samples serum with creatinine concentrations of

0.48 to 5.81 mg/dL. The within-run assay obtained CV=1.38% to 3.05%, and total assay obtained CV=2.95% to 6.84% on samples urine with creatinine concentrations of 0.73 to 235.12 mg/dL. Dilution linearity of multiple serum samples demonstrated means recoveries of 95.46% - 97.41%. Linearity test by applying eleven (11) points of dilution factors using the Standard Reference aqueous solution (SRM967) of the National Institute of Standards and Technology (NIST) at the following concentrations: 1.0 mg/dL and 30.0 mg/dL and the regression linear equation to this study was y = 0.956x + 0.057 (R^2=1). The comparative results between ADVIA Creatinine_2 and LABTEST Creatinine (Labtest Diagnóstica S.A) were performed in 53 serum samples and 40 urine samples; all known results were within the creatinine analytical measurement range. The obtained correlation coefficient to serum and urine samples was 0.999. The obtained linear regression to serum samples was 1.01384 to the ADVIA Creatinine_2 and -0.005066 to the LABTEST Creatinine. The obtained correlation coefficient to urine samples was 0.999. The linear regression was 0.987 to ADVIA Creatinine_2 and -0.52 to LABTEST Creatinine.

Conclusion: The results show that ADVIA Creatinine_2 test presents the total error estimate less than the allowable total error in all levels of clinical decision. The estimating bias are less than or statistically equal to the defined EQA Bias in a significance level of 2.5%. The evaluated results show that ADVIA Creatinine_2 test is an accurate method to measure creatinine serum and urine samples through a wide range of clinically relevant concentrations and it also shows equivalent performance to LABTEST Creatinine assay.

B-178

Serum creatinine determined by Jaffe and enzymatic method, in regular, icteric and hemolyzed samples

M. Friedrich. *Hospital Universitário, Universidade de São Paulo, São Paulo, Brazil*

Background: Serum creatinine is an important clinical marker for renal clearance. The Jaffe reaction remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations. With the recent introduction of the reporting of estimated glomerular filtration rate (eGFR), inter-laboratory agreement of serum creatinine results has become an important international priority. The aim of this study was to compare analytical performance and practicability of the enzymatic method and Jaffe method for serum creatinine for routine use and to compare the effects of some common interfering substances on both methods.

Materials and Methods: We assessed 221 serum samples obtained for routine clinical care: 106 regular samples (without interfering substances), 69 icteric and 57 hemolyzed samples. 11 samples were both icteric and hemolyzed. Serum creatinine was determined both by kinetic Jaffe's and enzymatic method on Siemens ADVIA Chemistry 1800. The ADVIA Chemistry Creatinine_2 (CREA_2) assay is a Jaffe, alkaline picrate, kinetic method and the ADVIA Chemistry Enzymatic Creatinine_2 (ECRE_2) assay is a Creatininase method. We analyzed the agreement between the two methods and determined the mean difference between them.

Results: Comparison of Jaffe (X) and enzymatic (Y) measurements of serum creatinine levels reveals significant correlation with or without the presence of interfering substances. However, mean differences between enzymatic to kinetic Jaffe's methods were higher for icteric and hemolyzed samples, as shown in the table below.

Conclusions: Serum creatinine can be overestimated by Jaffe's method in the presence of interfering substances, such as hemoglobin and bilirrubin. Enzymatic method is less affected by interferences so it is a better method to measure creatinine.

Table 1. Comparison between serum creatinine measured by Jaffe and enzymatic methods

	Mean (mg/dL)	Median (mg/dL)	SD	Lowest value (mg/dL)	Highest value (mg/dL)	R^2	Linear Regression	Bias
All samples (n=221)								
CREA_2 Jaffé	3.66	1.95	4.65	0.21	30.25	0.997	y = 1.023x - 0.21	-3.82
ECRE_2 Enzymatic	3.52	1.77	4.77	0.25	31.66			
Regular samples (n=106)								
CREA_2 Jaffé	4.85	2.46	5.90	0.21	30.25	0.998	y = 1.025x - 0.18	-1.23
ECRE_2 Enzymatic	4.79	2.27	6.10	0.25	31.66			
Icteric samples (n=69)								
CREA_2 Jaffé	2.52	1.8	2.62	0.46	15.17	0.992	y = 0.991x - 0.18	-8.33
ECRE_2 Enzymatic	2.31	1.58	2.60	0.51	15.05			
Hemolyzed samples (n=57)								
CREA_2 Jaffé	2.62	1.69	2.66	0.37	14.20	0.993	y = 0.997x - 0.20	-8.01
ECRE_2 Enzymatic	2.41	1.46	2.66	0.45	14.34			

B-179

The reference value of non esterified fatty acids determined by enzymatic method in healthy population

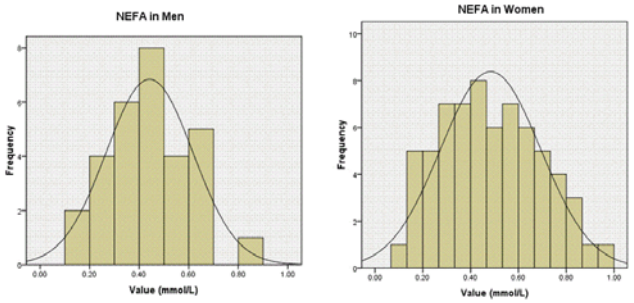
Y. Huang, T. Wang, X. Du, J. Jian, J. Kang, F. Chen, A. Zhu. *Beijing Strong Biotechnologies, Inc., Beijing, China*

Background: Increased circulating levels of nonesterified free fatty acids (NEFA) have been observed in such hyperinsulinemic states as obesity, impaired glucose tolerance, diabetes, and dyslipidemia where they have been causally linked to the development of insulin resistance and hyperinsulinemia.We created liquid enzymatic method for measuring NEFA. Appropriate reference ranges allow for effctive utilization of an assay. The aim of the current work was to establish NEFA reference ranges in healthy population.

Methods: Colleted normal serum specimens for referenc range determinations from 101 healthy individuals, age from 20 to 62 years, who attended Beijing Strong Biotechnologies for annual health check-up. None of these specimans contained Diabetes and hypertersion. Specimens containing high TG and high GLU were expected. The ration of female/male was 70/31, Blood was collected in the morning after fasting for 12 hours. Blood sample were stored on ice. Centrifugation was carried out within one hour. The serum samples were assayed on Hitachi 7180 within 2 hours of cellection. The outliers was excluded using Grubbs statistics mehtod.

Results: NEFA displayed a normal distribution for this reference population both male and female (Figure 1). The reference interval for NEFA using liquid enzymatic method provided different range between males and females, the range for females (n=70) was 0.02 to 1.02µmol/L and for males (n=31) was 0.10 to 0.78µmol/L

Conclusions: The reference range determined in this healthy population was gender dependent with higher levels for females.



B-180

Hydroxocobalamin Interference with Carboxyhemoglobin (COHb) Measurements

P. V. A. Pamidi, H. Yim. *Instrumentation Laboratory, Bedford, MA*

Background: *H*ydroxocobalamin (OHCbl), a vitamin B12 analog, is known to interfere with CO-Oximetry measurements (1). Recent study by Livshits et al (2) refers to two carbon monoxide poisoning patients and claimed that the CO-Oximeter reported falsely low carboxyhemoglobin after hydroxocobalamin therapy. The paper by Livshits et al went on to suggest that the false reading by the CO-Oximeter may lead to incorrect diagnosis and delay of appropriate treatment. However, assessment in out lab prior to this publication (1) showed a smaller interference at normal COHb levels. In this study, impact of hydroxocobalamin at high COHb levels is evaluated.

Methods: Blood samples collected from healthy donors were used to prepare COHb levels (25 -50 %). COHb samples spiked with hydroxocobalamin (1 g/L) were measured on 3 GEM Premier 4000 analyzers. The effect of OHCbl interference on carboxyhemoglobin is evaluated using the measured difference between the unspiked and the spiked samples. To emulate the treatment conditions reported in Livshits reference, blood samples were tonometered with 100% oxygen.

Results: Carboxyhemoglobin data shown in table below confirmed that accuracy is slightly affected by the presence of OHCbl (1-2 units). However, with or without OHCbl, the reduction in COHb is mainly triggered by the oxygen treatment. In addition, spiked samples were appropriately flagged by the iQM software.

Conclusions: Blood samples spiked with 1 g/L OHCbl showed a small interference (1-2%) on carboxyhemoglobin consistent with our prior data in reference 1. Based on these results, the dramatic reduction in the COHb level as reported by Livshits is not due to interference from hydroxocobalamin and oxygen treatment would likely have caused reduction in COHb levels.

References: 1) P.V.A. Pamidi, et al , Clin. Chim. Acta, 401, 2009, 63-67.

2) Z. Livshits, et al, New ENGLAND J. MED 367: 1270 - 1271, SEPTEMBER 27, 2O12

COHb Sample	COHb	COHb + OHCbl 1 g/L	Delta
25% COHb	26.7	25.8	0.9
25% COHb (O_2 Tonometry 0.5 hr)	19.9	20.2	-0.3
25% COHb (O_2 Tonometry 1 hr)	14.3	15.0	-0.7
25% COHb (O_2 Tonometry 2 hrs)	7.3	7.7	-0.4
25% COHb (O_2 Tonometry 3 hrs)		3.7	
50%COHb	52.3	50.7	1.7
50%COHb (O_2 Tonometry 0.5 hr)	41.1	38.1	3.0
50%COHb (O_2 Tonometry 1 hr)	28.9	27.5	1.3
50%COHb (O_2 Tonometry 2 hrs)	15.3	12.3	2.9
50%COHb (O_2 Tonometry 3 hrs)		5.7	

B-181

Demonstration of In-Vitro Synthesized Calcium Oxalate Dihydrate Crystals with Native Octahedral Morphology for Use in Urine Sediment Controls

B. Fernández, C. Grandjean, M. Ghadessi, M. Ban. *Quantimetrix, Redondo Beach, CA*

Background: The microscopic analysis of urine sediment is routinely performed to screen for the presence of red and white blood cells, epithelial cells, microorganisms, casts, and a wide range of crystals. Crystals, regardless of type, result from the precipitation of urine solutes out of solution. When crystals are found in freshly voided urine, they indicate formation in-vivo and are usually clinically significant. Factors contributing to crystal formation include the concentration of the solute in the urine, pH, and flow rate of urine through the renal tubules. Super-saturation can arise from dietary excess, dehydration, or from certain medications. Oversaturation of urine with crystals can lead to stone formation causing pain and an increased incidence of urinary tract infections. Nearly 80% of all urinary stones are calcium compounds, especially calcium oxalate. Calcium oxalate dihydrate (COD) crystals in an octahedral morphology are the most commonly observed crystals in human urine. Urine sediment control materials, used by clinical labs to validate the processing, centrifugation, and microscopy of urine samples, would therefore ideally contain the native octahedral form of COD crystals.

Objective: To synthesize calcium oxalate dihydrate (COD) crystals with the native octahedral morphology for use in urine sediment control formulations.

Methods: Two reagents were prepared: Reagent A was an aqueous solution of sodium oxalate at 1mg/mL and Reagent B was an aqueous solution calcium chloride at 4 mg/mL with other proprietary excipients. One part of Reagent A was slowly added to four parts of Reagent B under very controlled processing conditions. The resulting COD precipitate was centrifuged and the supernatant was decanted. The pellet was re-suspended in water then spiked into the Quantimetrix Dip&Spin® and QuanTscopics® urine sediment control formulations for evaluation under standard brightfield microscopy and on the IRIS IQ®200 automated urine sediment analyzer.

Results: Brightfield microscopy at 400x magnification showed a majority of COD crystals with near perfect octahedral, bi-pyramidal morphology at various sizes ranging from 5-20 µM in diameter in a single preparation. Calcium oxalate monohydrate (COM) crystals in an ovoid morphology were also apparent to a lesser degree in the same preparation. Different preparations made under non-optimal processing conditions resulted in very large COD crystals with complex four-armed cruciform, stellate, or cloverleaf morphologies. The optimized COD crystal preparation in both the Dip&Spin® and QuanTscopics® formulations were accurately identified and characterized by the IRIS IQ®200 analyzer.

Conclusion: This novel method for synthesizing COD crystals with the native octahedral morphology identical to the most commonly observed crystal type found in human urine makes for an ideal urine sediment control. Automated urine sediment systems like the IRIS IQ®200 and 77 Elektronika UriSed® analyzers have been particularly challenging as they do not always recognize the presence of crystals in some third party control formulations. The new Quantimetrix Dip&Spin® and QuanTscopics® formulations with the improved COD crystals are easily characterized by both standard microscopy and automated microscopy methodologies making for an excellent control solution for the clinical lab.

B-182

Reference Intervals for Random Urinary Calcium and Magnesium

V. Gounden, C. Y. Park, T. Prabhala, K. Spaid, S. J. Soldin. *National Institutes of Health (Clinical Center), Bethesda, MD*

Background: To estimate the rate of excretion of urinary constituents, a 24 hour sample is required. However these are cumbersome for the patient and often are not collected accurately. The use of the analyte ratio to urine creatinine accurately reflects the 24 hour excretion as creatinine is excreted at a constant rate throughout the day. At the Department of Laboratory Medicine, NIH Clinical Center reference intervals for urine calcium:creatinine and urine magnesium: creatinine ratios based on the hospital population were previously unavailable. Thus it was difficult to interpret random urine results for calcium and magnesium and this required that a timed or 24 hour specimen be collected for accurate interpretation.

Objectives: To determine the reference intervals for urinary calcium:creatinine and urinary magnesium : creatinine ratios using the Hoffmann method (Hoffmann RG Statistics in the practice of medicine JAMA 1963;185:864-73) at the National Institutes of Health Clinical Center

Methods: Data was collected for a total of 159 individuals between the ages of 4-73 years. This study population consisted of 131 healthy outpatients seen at the NIH Clinical Center and 28 normal volunteers. Data analysis was performed on urine samples collected between May and July 2013. Outpatient data for urine calcium, magnesium and creatinine results as well as demographic details were obtained from the laboratory information system (SoftLab®, SCC Soft Computer ,FL). The Dimension XPand chemistry analyzer (Siemens Diagnostics,Tarrytown NJ) was used to measure the concentrations of urine creatinine, calcium and magnesium following the manufacturer's guidelines. All analytes are measured by spectrophotometric, bichromatic rate technique.

Urine creatinine, magnesium and calcium values were converted to SI units (mmol/l) before further data analysis. Statistical analyses were performed on Microsoft Excel. The ratios were ranked from smallest to largest values. Then, we developed a percent cumulative frequency chart and plotted the cumulative percent frequencies against the log of these ratios/values. Using the Hoffmann approach, we analyzed the linear portions of the curve and calculated the line of best fit. We calculated the 2.5th and 97.5th percentile values as the new reference intervals of these analytes.

Results: The following reference intervals were found employing the Hoffman approach. Urine calcium:creatinine ratio was 0.11 - 1.03 mmol/mmol and for urine magnesium: creatinine the ratio was 0.20-0.76 mmol/mmol.

Conclusions:The availability of reference intervals for urine calcium: creatinine and urine magnesium:creatinine ratio allow for random specimens to be better utilized for clinical and diagnostic purposes. This is also more convenient for the patient and health care providers. An interesting question for future studies revolves around possible gender and racial disparity of these reference intervals and the influence of seasonal changes on these ranges.

B-183

Reference range for sodium, potassium and chloride in single spot urine samples

A. C. C. S. Botelho[1], W. Pedrosa[1], L. S. Vasconcellos[2], S. M. Eloi-Santos[2]. *[1]Hermes Pardini, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: The determination of urinary electrolytes is of great importance in the investigation of systemic metabolic disorders. Since renal excretion daily rate is not uniform during the day, the 24-hour urine is considered the gold standard biological sample. However, its replacement by a fresh urine sample could reduce patient discomfort and the possibility of pre-analytical interferences. Nevertheless the findings in the literature are conflicting. This study aimed to estimate the pattern of excretion of sodium, chloride and potassium over 24h and define reference ranges for the periods presenting good correlation with the 24-hour urine.

Method: Timed sequential urine samples were collected from 41 healthy adults, aged 18-60 years in the following periods: 6am-9am (after breakfast), 9am-12pm, 12pm-3pm, 3pm-6pm, 6pm-9pm and 9pm-6am. Sodium, potassium and chloride were measured by Ion Selective Electrode method and creatinine by Kinetic Colorimetric method (Roche P- Modular®). The values of each sample were correlated with those obtained from the 24-hour urine. The urine samples from the best correlated periods were used to establish the reference ranges, following Clinical and Laboratory Standards Institute (CLSI). For that, samples of 120 healthy subjects were used and confidence interval was 95% and the significance level of 0.05.

Results: For sodium and chloride, high positive correlation was seen in all period samples. However, the best correlation was obtained with samples collected between 6am-9am, after breakfast (sodium: r=0.6185, p=0.0028; chloride: r=0.5787, p = 0.0060). For potassium measurement, the best correlation was with urine collected between 6pm-9pm (r=0.5824, p=0.0001). The reference ranges are shown in Table 1.

Conclusion: It is possible to replace the 24-hour urine sample by spot urine collected in predetermined periods: 6am-9am for sodium and chloride and 6pm-9pm for potassium.

Reference range for sodium, potassium and chloride in single spot urine samples

Electrolytes	Time of collection	Lower limit (mEq/g creatinine)	Higher limit (mEq/g creatinine)
Na	6am-9am	24 (90% CI 15.0 to 33.7)	300 (90% CI 229.8 to 309.8)
Cl	6am-9am	19 (90% CI 12.8 to 36.6)	287 (90% CI 275.2 to 337.3)
K	6pm-9pm	11 (90% CI 10.1 to 14.1)	91 (90% CI 74.2 to 108.6)

B-184

A Comparison of Creatinine Measurement by the Jaffe and Enzymatic Methods in an Outpatient Population

A. H. Adams, J. A. Straseski, C. M. Lehman, R. L. Schmidt. *University of Utah and ARUP Laboratories, Salt Lake City, UT*

Background: Serum creatinine (SCr) concentrations and estimated glomerular filtration rates (eGFR) are widely used for the evaluation of renal function. The Jaffe and enzymatic methods are the most common methods for creatinine measurement. The Jaffe method is generally less expensive than the enzymatic method but is more susceptible to interferences. Significant savings could be obtained if populations could be identified where the interference rate of the Jaffe method is acceptably low. Most studies on Jaffe interferences have used spiked samples and the rate of interferences in defined patient populations has not been well characterized. The interference rate is likely to vary by patient population. The objective of this study was to compare the creatinine and eGFR results from Jaffe and enzymatic creatinine methods in an outpatient population.

Methods: This study analyzed 545 unique, randomly selected, outpatient samples over a period of 45 days. Samples were analyzed using both the Jaffe (kinetic alkaline picrate, Abbott Laboratories) and enzymatic (Creatininase, Abbott Laboratories) methods using an Abbott Architect c8000. eGFRs were calculated using the CKD-EPI equation. A 20 day precision study, following CLSI guidelines, was also performed that evaluated both creatinine methods at concentrations of 0.28, 0.79, 1.21, 2.73, and 5.08 mg/dL.

Results: Orthogonal (Deming) regression showed no significant difference between the Jaffe and enzymatic methods. The slope was 1.006 (95% CI: 0.998, 1.103) and the intercept was -0.005 (95% CI: -0.015, 0.006). The average difference (bias) between the methods was -0.007 mg/dL. The Bland-Altman (BA) limits of agreement (LOA) for the creatinine difference were -0.139 and 0.136 mg/dL. The Bland-Altman limits of agreement for the CKD-EPI eGFR were -10.3 and 10.4 mL/min. 3.1% (17 of 543) of CKD-EPI eGFR discrepancies resulted in a change of classification with respect to the 60 mL/min decision limit. The difference between the predicted and actual CKD-EPI discordance at the 60 mL/min decision limit was not statistically significant (chi sq= 0.03, p=0.86). The Jaffe method performed with greater precision at four of the five concentrations in the 20 day precision profile. At concentrations of 0.28, 0.79, 1.21, 2.73, and 5.08 mg/dL, the CVs of the Jaffe method were 3.0%, 1.4%, 0.8%, 0.8% and 0.8%, respectively. The corresponding CVs of the enzymatic method were 2.9%, 1.7%, 1.7%, 1.3%, and 1.2%.

Conclusion: At our institution the Jaffe method generally had greater precision than the enzymatic method. Discrepancies in the CKD-EPI eGFR based on the Jaffe method did not result in a statistically significant increase in disease reclassifications at the 60 ml/min decision limit in an outpatient population. Studies are needed to characterize the relative rate of interference in additional populations.