Tuesday PM, July 30, 2002

Poster Session: 2:00 pm - 4:30 pm
Factors Affecting Test Results

B-51

Urinary creatinine measurement using a Vitros 250 chemistry analyzer compared with the Jaffe method. E. A. Knecht, E. F. Krieg, Jr, J. C. Clark, J. S. Kesner, *National Institute for Occupational Safety and Health, Cincinnati, OH.*

Creatinine forms in muscle as a waste product from creatine and phosphocreatine, and is excreted in urine at relatively constant rates. This allows creatinine excretion to be used as a check for the completeness of 24-hr urine collections, and to normalize, for urine flow rate, urinary concentrations of endogenous and exogenous substances primarily excreted by renal filtration. Because urinary creatinine levels are 10-50 fold higher than those measured in serum, urine samples are typically diluted prior to creatinine analysis. The Vitros 250 chemistry analyzer (Ortho Clinical Diagnostics, Rochester, NY, USA) measures creatinine using a single-slide enzymatic method comprised of a dry, multilayered analytical element coated on a polyester support. The Vitros employs three levels of standard to calibrate creatinine. The highest standard (17 mg/dL) corresponds to 340 mg/dL for urine diluted 20-fold, the dilution recommended by Ortho as a basis for reference accuracy.

Recently, as part of a large national collaborative health study, our laboratory measured creatinine in 653 urine samples collected, primarily, by men (n=335). The proportion of samples with creatinine levels above 200 mg/dL were more than twice that seen in three previous population studies. Samples with creatinine concentrations exceeding the upper limit for the Vitros standard curve for creatinine (340 mg/dL) were re-assayed at higher dilutions, accompanied by urine samples with creatinine concentrations within the assay range to confirm repeatability. While Performance Verifier (serum matrix) values were similar between the slide lots used in this study, Bio-Rad urine pools and within-range urine samples decreased 4-8% ($p < 0.005$) with the new slide lot used to analyze the final group of urine samples. This lot-to-lot variability was adjusted based on Bio-Rad urine pool values.

Creatinine concentrations increased when urine samples were diluted beyond 1:20. This occurred in samples measured at multiple dilutions within the same assay, and principally among samples with higher creatinine concentrations. Segmental model analysis revealed deviations from parallelism ($p<0.05$) for urinary creatinine concentrations exceeding 94-130 mg/dL when sample dilutions increased from 1:20 to 1:30 or 1:40. Although Vitros values strongly correlated ($r=0.995$, $p=0.001$) with values derived from the same samples measured by the Jaffe method, segmental model analysis revealed statistically significant differences in creatinine measurements between the two methods. Deviations from parallelism ($p<0.05$) occurred between the two methods at 112 mg/dL at a 1:20 Vitros dilution, and at 214 mg/dL at a 1:30 Vitros dilution. At a 1:40 dilution, Vitros data increased ($p<0.05$) until 295 mg/dL, then decreased ($p<0.05$) in comparison to Jaffe data.

These results indicate that Vitros creatinine measurements deviate from parallelism as urinary creatinine concentrations increase. When Vitros data were compared to creatinine concentrations measured by the Jaffe method, greater agreement was found in urine analyzed at dilutions higher than the 1:20 dilution recommended by the manufacturer. Our results suggest that concentrated urine samples exert a matrix effect on the Vitros method, and that dilutions of 1:30 or 1:40 may be more appropriate for creatinine analysis in urine samples.

B-52

Evaluation of the effects of urine turbidity on DCA 2000 microalbumin/creatinine assays. C. E. Hiar[1], P. C. Painter[2], D. R. Parker[1], Y. Jubron[2], D. Wilson[2], [1]*Bayer Corporation, Elkhart, IN,* [2]*University of Tennessee Medical Center, Knoxville, Knoxville, TN.*

Early detection of microalbuminuria can result in medical interventions that prevent or slow onset of serious renal disease. The DCA 2000+ ® System is an effective tool for accurately measuring microalbuminuria; with an easy-to-use cartridge format for albumin, creatinine, and A:C ratio quantification in 40 µL of random or timed urines. Elimination of the centrifugation step for urine clarification, simplifying the albumin and creatinine assays even more, would be advantageous. DCAs are programmed to give error messages when specimen blank absorbance exceeds 0.150 mA, avoiding any negative influence of turbidity on system results. Study objectives were: 1) assess the effects of increased turbidity caused by urine particulates on DCA 2000+ methods and 2) demonstrate that error messages are obtained when specimen blank readings exceeded the absorbance limits.

Laboratory staff collected 89 fresh, turbid urines. Particulate matter was characterized using a Yellow Iris Analyzer. Turbidity ranged from "clear" (no turbidity) to "very cloudy" or "turbid". Specimens contained the common particulates found in urines and were analyzed in duplicate. Data collection software captured DCA blank absorbance readings. Urines had to be concentrated by centrifugation to achieve high blank readings. Two microalbumin/ creatinine controls were included in each DCA run to confirm acceptable performance. Data analysis included comparisons of results obtained before and after centrifugation for albumin, creatinine and A:C ratios. Three statistical comparisons were performed (results from non-concentrated urines, concentrated urines and "all" results). Statistics were:

Albumin ("all"); y=1.026x -0.300 mg/L, r=0.997, Syx=5.9, N=184, median bias=(-)0.2%;

Creatinine ("all"); y=0.979x -0.003 mg/dL, r=0.996, Syx=8.3, N=209, median bias=(-)1.6%;

A:C ratio ("all"); y=0.996x +0.200 mg/g, r=0.998, Syx=4.9, N=180, median bias=0.0%.

T-tests of microalbumin, creatinine and A:C ratio comparisons, before and after centrifugation, demonstrated that results from centrifuged urines (clarified) were equivalent to results of non-centrifuged urines. For urines with a blank absorbance of ≤ 0.15 mA, microalbumin and creatinine results were always obtained. With urine blank absorbances of > 0.15 mA, error messages were always obtained. These data demonstrated reliable DCA System performance in detecting urines with absorbance blanks higher than the pre-set limit.

No effect of turbidity on DCA 2000+ microalbumin, creatinine and A:C ratio results was detected, confirming that specimen centrifugation is unnecessary. The study also demonstrated that DCAs always gave an error message for urines with blank absorbances greater than the programmed limit (>150mA). This "fail safe" mechanism ensures that, for excessively turbid urines, microalbumin and creatinine results will not be reported.

B-53

Interference evaluation of the VITROS 5,1 FS Chemistry System MicroSensor™ (sample integrity) module. K. L. Warren, B. Sawyer, S. Stearns, T. Cheehan, G. Merrill, G. Mehalek, C. Li, C. Least, Jr., *Ortho-Clinical Diagnostics, Rochester, NY.*

Sample integrity (SI) analysis is used by clinical laboratories to evaluate pre-analytical specimen quality. SI is defined as the degree of hemolysis, icterus and/or turbidity (lipemia) in a specimen and is often a very subjective, operator dependent, visual assessment, reported as "sample indices'. The VITROS 5,1 FS Chemistry System (currently in development) introduces an automated spectrophotometric reagent-less method for determining specimen quality. Called MicroSensor, it automatically evaluates the suitability of each sample in terms of hemolysis, icterus and turbidity for analysis. The objective of this study was to evaluate the effect of potentially interfering substances in clinical specimens on the performance of the MicroSensor feature.

A "paired difference' method, as described in NCCLS Document EP-7 Interferent Testing, was used to test over 20 compounds that could occur in patient sera. Emphasis was placed on testing diagnostic dyes, contrast media and highly colored drugs and vitamins. A high concentration of each compound was spiked individually into a serum pool. The serum pool was spiked approximately to a hemoglobin of 50 mg/dL, icterus at 1.5 mg/dL and turbidity (Intralipid) at 40 mg/dL. Testing was performed on the VITROS 5,1 FS Chemistry System using the MicroSensor feature. Compounds exhibiting a bias < 20% were not considered to interfere. Compounds exhibiting a bias > 20% were considered to interfere.

The following compounds, when tested at the levels indicated, were found to cause biases as shown below using the serum pool.

Compound	Classification	Test Level	Hemoglobin % bias	Icterus % bias	Turbidity % bias
Amrinone	Colored compound	1.0 mg/dL	0.1	-1	2
Chloroquine	Colored compound	2 uM/L	-0.3	-1	-1
Diphenhydramine	Colored compound	10 ug/mL	-1	4	2
Doxorubicin	Colored compound	18 ug/dL	0.2	-2	-3
Hypaque	Radiographic contrast media	0.6 I / dL	0.3	12	-5
Novobiocin	Colored compound	15 mg/dL	0.4	9	3
Primaquine	Colored compound	300 ng/mL	1	1	14
Quinacrine	Colored compound	200 ng/mL	1	0.4	3
Triamterene	Colored compound	6 mg/dL	-0.5	-3	-17

Supplement to

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Clinical Chemistry

International Journal of Laboratory Medicine and Molecular Diagnostics

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