

Health ABC sub study analyte measurement methods

Urine IL-18 was measured using a commercially available ELISA kit (Medical & Biological Laboratories Co., Nagoya, Japan) per manufacturer's instructions (intra and interassay CV = 7.2/7.5% respectively). (Krawczeski CD, Goldstein SL, Woo, JG, Wang Y, Piyaphanee N, Ma Q, Bennett M, Devarajan P. Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass. *Journal of the American College of Cardiology*, 2011, 58:2301-2309.) The urine KIM-1 ELISA was constructed using commercially available reagents (DuoSet DY1750, R & D Systems, Inc., Minneapolis, MN) as described previously (intra and interassay CV = 5.6/4.9). (Chaturvedi S, Farmer T, Kapke GF. Assay Validation for KIM-1: human urinary renal dysfunction biomarker. *Int J Biol Sci* 2009; 5:128-134). The Microalbumin (MALB) assay is based on a particle-enhanced turbidimetric inhibition immunoassay (PETINIA) adapted to a clinical chemistry system which allows direct quantitation of albumin in urine samples (Siemens, Newark, DE). The analytical sensitivity of the MALB assay is 1.3 mg/L (intra and interassay CV = 2.3/6% respectively). Creatinine was measured by a modified Jaffe method (Larsen, K. Creatinine assay by a reaction-kinetic approach. *Clin Chem Acta* 1972; 41: 209-217) on a clinical chemistry analyzer (Siemens, Newark, DE). The analytical sensitivity of the creatinine assay is 0.05 mg/dl (intra and interassay CV = 0.6/1.1% respectively).

Urine electrolytes were measured using the Quiklyte Integrated Multisensor on a Siemens Dimension Xpand plus HM clinical analyzer (Siemens, Munich, Germany). Intra and inter-assay CVs were 0.73 and 1.6% respectively. Urine Alpha-1 microglobulin were measured by immunonephelometry on a Siemens BNII clinical nephelometer. Intra and inter-assay CVs were 4.1 and 10.3% respectively. The urine NGAL ELISA was performed using a commercially available assay (NGAL ELISA Kit 036; Bioporto, Grusbakken, Denmark) that specifically detects human NGAL (Bennett et al., 2008). The intra-assay coefficient of variation (cv's) was 2.1% and inter-assay variation was 9.1%. Uromodulin was measured with a commercially available assay (Uromodulin Glycoprotein ELISA, M036020, mdbioproducs, Zurich, Switzerland) per manufacturer's instructions. Intra and inter-assay CVs were 8.4 and 11.6%, respectively.

Sodium, Potassium and Chloride were measured on a Dimension Xpand plus HM Clinical Analyzer (Siemens, Munich, Germany). The assay uses integrated multisensory technology to develop an electrical potential proportional to the activity of each ion in the sample. The assay range is 5-300 mmol/L for sodium (intra/interassay CV's 0.5/1.2%), 1-300 mmol/L for potassium (intra/interassay CV's 0.7/1.9%), and 10-330 mmol/L for Chloride (intra/interassay CV's 0.8/1.2%).

Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, Syed H, Ali S, Barasch J, Devarajan P (2008) Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clinical journal of the American Society of Nephrology* : CJASN 3:665-673.