SUPPLEMENT

COMPLETE METHODS

Multiplex Assay for Kidney/Tubular Injury Markers

Seven different tubular injury markers were measure in urine samples using the Kidney Injury Panel multiplex kit from Meso Scale Discovery (MSD, Rockville MD), according to the manufacturer's instructions. The markers were albumin, beta-2-microglobulin (B2M), cystatin C, osteopontin (OPN), epithelial growth factor (EGF), uromodulin (UMOD), and neutrophil gelatinase-associated lipocalin (NGAL). The multiplex nature of this assay is based on the coating of a capture antibody for each analyte at a different position in each well of a 96 well plate. After washing and blocking the plate, urine samples, diluted 1/500 in MSD sample diluent (see below), were added to the wells and incubated for 2 hours at room temperature. The samples were removed, the wells were washed thrice, and a mixture of seven antibodies, one for each marker and each containing a chemiluminescent label, was added and incubated further for 2 hours at room temperature. Each detection antibody contains. After the labeled antibodies are removed by washing, a MSD reading buffer was added, and the plates were read for light intensity in an MSD Spector Imager after applying voltage to cause the chemiluminescent label to emit light, the intensity of which is proportional to the amount of detection antibody. Wells with standard concentrations of purified marker were run for each tubular marker, and the amount of each marker in the urine samples was calculated by regression analysis. Diluent was used as negative control.

At the time of the assay, the urine samples were quickly thawed from -80^{0}C in room temperature water, immediately cooled on ice, and then centrifuged at 16xg for 10 minutes. Each urine was sampled from the top of the supernatant, diluted, and added immediately to the MSD plate.

Urine analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Ten ul of each urine sample was added to 10 ul of NuPage 4X LDS buffer (Invitrogen Life Technologies, Thermo Fisher Scientific, Waltham, M), the mixtures was heated at 90^{0}C for 5 min, cooled to room

temperature, loaded on to NuPage 4-12% Bis-Tris SDS-PAGE gels, and electrophoresed at room temperature for 30 minutes at a constant 200 volts. Each gel was rinsed thrice, each time for 5 minutes in 200 ml of distilled water, and then stained for protein bands using Imperial Protein Stain (Thermo Scientific) according to the manufacturer's instructions.

For testing the effects of 0.5% acetic acid, 9 volumes of urine were mixed with 1 volume 5% acetic acid, or 1 volume of water as control, and incubated at room temperature for various times, and then analyzed by SDS-PAGE as described above.