Creatinine

Version: 1
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Summary: Procedure followed to detect the concentration of creatinine in serum, plasma, and urine.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Acetonitrile	J.T. Baker	9012-03
LC/MS/MS Buffer		
D3-Creatinine	CDN Isotopes	W212P16

Protocol:

1. Fill 1.5mL eppendorf tubes with 80µL of acetonitrile.
2. Add sample:
    a. Use 20µL of plasma or serum/tube
    b. Use 6 µL of urine/tube
3. Add 6.5 µL of d3-creatinine (5mg/dl) to each tube.
4. Vortex each sample for 5 seconds.
5. Spin each sample at 4˚C for 10 minutes
6. Transfer supernatant to LC/MS/MS vials.
7. Dry samples in speed vacuum.
8. Resuspend pellet in 75µL of LC/MS/MS Buffer

9. LC/MS/MS (liquid-chromatography/ tandemmass spectrometry) analysis:
LC- Column: Isocratic using a Hamiltion PRP-X200 column. Mass Spectroscopy: MRM mode with parent/daughter ion pairs of 114/44 for creatinine and 117/47 for d3-creatine. (Ref: Takahashi N, Boysen G, Li F, Li Y, Swenberg JA. Tandem mass spectrometry measurements of creatinine in mouse plasma and urine for determining glomerular filtration rate. Kidney Int. 71: 266-271, 2006.)

Reagent Preparation:

Reagent 1: Acetonitrile: As supplied by vendor
Reagent 2: LC/MS/MS Buffer: 85% water and 15% of 8.5mM Ammonium Acetate.
Reagent 3: D3-Creatinine standard: (5 mg/dL)