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Protocol status: Working
 We use this protocol and it's working

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 21285

Yale - Blood and Urine Creatinine

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ABSTRACT

Summary:

Procedure followed to detect the concentration of creatinine in serum, plasma, and urine.

MATERIALS

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⊗ Acetonitrile **J.T. Baker Catalog #9012-03**

⊗ LC/MS/MS Buffer **Contributed by users**

⊗ D3-Creatinine **CDN Isotopes Catalog #W212P16**

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)

Keywords: creatinine

- 1** Fill 1.5mL eppendorf tubes with 80 μ L of acetonitrile.
- 2** Add sample:
 - a. Use 20 μ L of plasma or serum/tube
 - b. Use 6 μ L of urine/tube
- 3** Add 6.5 μ L 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 75 μ L of LC/MS/MS Buffer

9 LC/MS/MS (liquid-chromatography/ tandemmass spectrometry) analysis:

LC- Column: Isocratic using a Hamilton 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.*)