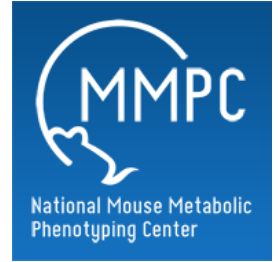


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Yale - Blood and Urine Creatinine

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Protocol status: Working

We use this protocol and it's working

Created: March 11, 2019

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Keywords: creatinine, urine creatinine summary, concentration of creatinine, urine, serum, yale

Abstract

Summary:

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

Materials

MATERIALS

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

 LC/MS/MS Buffer

 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)

Troubleshooting

- 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.)*