

**PLASMA UREA NITROGEN**  
**Updated September 2013**

I. Personal Protective Equipment:

- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves

II. Sigma Procedure: 63-UV Cat.#:

A. Using a 96 well microtiter plate (Ranin 96-CPF-1CS; ID = .638cM, Vol = 350uL), add 8  $\mu$ L of Serum/Plasma sample and Controls (Pooled serum, Abnormal/Normal Accutrol – Sigma #A3034 & A2034) in duplicate to corresponding wells.

B. Immediately add 200  $\mu$ l of BUN Reagent to all wells.

C. Place in microtiter plate reader (Dynatech Labs.).

1. Set absorbance at 340nm

2. Temperature = 37<sup>o</sup> C

3. Readings and interval = 9 every 15 sec with 5 sec shake prior to 1<sup>st</sup> reading

4. Determine  $\Delta A/\text{min}$  on readings 5 – 9 using OD/min with Linear Regression

D. Calculate PUN concentrations as follows:

- 1. PUN mg/dL = ( $\Delta A/\text{min}$  Unknown /  $\Delta A/\text{min}$  Calibrator)\*Conc. of Calibrator or
- 2. Use standard curve developed from Sigma Calibrators