Name  Blood Urea Nitrogen  
(Diacetyl Monoxime)

Summary and Principle
Urea is the principle waste product of protein catabolism. It is synthesized in the liver from ammonia which is produced as a result of the deamination of amino acids. Normally, urea nitrogen in the blood comprises only about 45% of the non-protein nitrogen. The diacetyl monoxime methodology for BUN determination is direct and measures a chromogen formed from the condensation of urea with diacetyl. This condensation methodology does not suffer from ammonia interference and utilizes less caustic reagents than other methods. Diacetyl monoxime is hydrolyzed under acidic conditions to produce diacetyl which then condenses with urea to form a pink chromogen that is measured at 520 nm. Thiosemicarbazide and ferric ions are employed to enhance the color development.

Clinical Significance
While a high protein diet, administration of cortisol-like steroids and stressful situations can increase the concentration of BUN, the test is generally used to detect prerenal, renal, and post-renal malfunction. increases in BUN are encountered with acute and chronic nephritis, intestinal and urinary obstruction, metallic poisoning, pneumonia, uremia, Addison's disease, peritonitis, surgical shock and cardiac failure. The BUN concentration is low in late pregnancy when the fetus is growing rapidly and utilizing material amino acids, in starvation, and in patients whose diet is grossly deficient in protein. decreased in BUN are also noted in nephrosis, acute liver destruction, and amyloidosis

Normal Values
Although each laboratory should establish their own normal range, results of 7.0-18.0 mg/dL are reported by some authors.

Acceptable Student Result Range
Patient samples should be within normal value range. Abnormal values should be brought to the attention of an instructor. Control samples must be within ± 2 SD.

Time Frame
60 minutes

Specimen
Serum: Blood is drawn into a tube which does not contain anticoagulant and is allowed to clot. Serum is separated from clot by any approved method and may be stored for several days refrigerated, or for several months frozen, without appreciable loss of urea.
Plasma: Add whole blood directly to a tube containing anticoagulant. Most common anticoagulants may be employed. Centrifuge to obtain plasma.

Urine: Urine may be tested for urea nitrogen (test is technically referred to as urine urea nitrogen UUN). Urine should be assayed within several hours following collection. Refrigeration is recommended to prevent bacterial decomposition of urea. Stability may be prolonged by freezing or by prior addition of glacial acetic acid (5 ml) to vessel used for a 24-hour specimen. Interfering Substance: It is advised that the analysis be performed on fresh non-hemolyzed serum to prevent loss of urea to bacterial contamination. Moderate hemolysis (0.15 g dL Hgb), bilirubin levels up to 20 mg/dL, and moderate lipemia do not cause significant interference with this method.

Reagents
1. BUN Color Reagent
diacetyl monoxime 16.6 mmol/L

   BUN Color Reagent should be clear to pale yellow solution. Darkening or formation of a precipitant in the reagent would indicate contamination and the reagent should be discarded.

   Caution: Do not take internally. In if ingested, perform gastric lavage. Call a physician. Avoid contact with skin, eyes, or clothing. Flush affected area with water and seek medical attention.

2. BUN Acid Reagent
   ferric chloride 0.21 mmol/L
   phosphoric acid 0.01 mol/L
   sulfuric acid 1.9 mol/L

   The BUN Acid Reagent should be a clear colorless solution. Failure to achieve assay values on freshly prepared control sera may indicate reagent deterioration.

   Poison: May be fatal if swallowed. Seek medical advice. If taken internally, do not give emetics or baking soda. External contact: Wash with sodium bicarbonate. Eye contact: flush with water and call a physician.

3. Urea Nitrogen Standard Solution
   Store in refrigerator at 0-5°C.

Materials Required But Not Provided
1. Spectrophotometer or suitable instrument calibrated to read absorbance at 520 nm.
2. Test tubes and Cuvets
3. Pipettes: 0.02 mL (20μL), 1.5 ml, & 3 ml
4. Timer or stopwatch.
5. Waterbath or heatblock capable of maintaining 100°C.

**Linearity**

Linearity extends to 150 mg/dL. Samples exceeding 150 mg/dL should be diluted with saline and repeated. Multiply results by dilution factor when calculating the unknown.

**Procedure**

1. Label three (3) or more test tubes or cuvettes Blank, Standard, Test 1, Test 2, etc.
2. To each, add 1.5 mL BUN Color Reagent.
3. To Blank, add 20 μL water.
   To Standard(s) add 20 μL Urea Nitrogen Standard Solution.
   To Test add 20 μL serum or plasma
   Mix contents by gently swirling.
4. Add 3.0 mL BUN Acid Reagent into all tubes, mix well.
5. Incubate for 10 minutes at 100°C in a heat block or 8 minutes in a boiling water bath.
6. Remove all tubes and cool to room temperature using cold tap water.
7. Mix all tubes prior to reading the absorbance.
8. Set the wavelength of the spectrophotometer at 520 nm. Zero with the Reagent Blank.
9. Read and record the absorbance of controls and patient samples. Determine concentration by graph or calculation as instructed. Final color is stable for 6 hours at controlled room temperature.

**Calculations**

**Quality Control**

Two levels of assayed control, ie. Monitrol I and Monitrol II

**Reference**

DMA BUN, Diacetyl Monoxime Procedure No. 1750