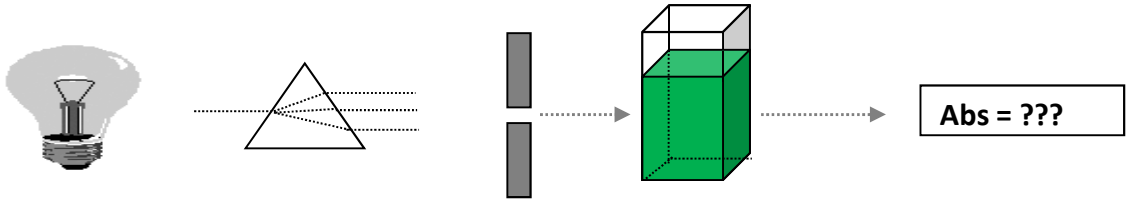
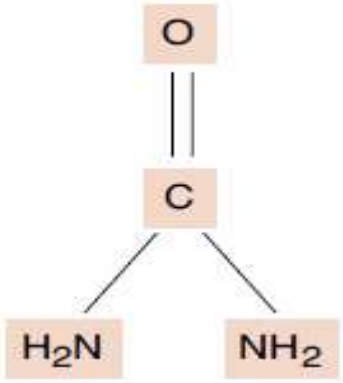


Assay for Urea

Yoseph Cherinet (BSc, MSc, Asst. Prof)



Biochemistry Lab

Rationale

- Urea is product of amino acid breakdown in the liver & readily filtered from the plasma by the glomerulus.
- Decreased renal function, decreased blood flow to the kidneys causes an increase in plasma urea concentration as a result of compromised urea excretion.
- Decreased plasma urea concentration include low protein intake, severe liver disease and during late pregnancy as a result of increased protein synthesis.

Specimen requirements & interfering substances

- Plasma /serum or urine can be used
  - Serum is recommended for the assay. Plasma may also be used, provided that the anticoagulant used contains neither ammonium nor fluoride salts. Fluoride inhibit urease & ammonium ions interfere the test.

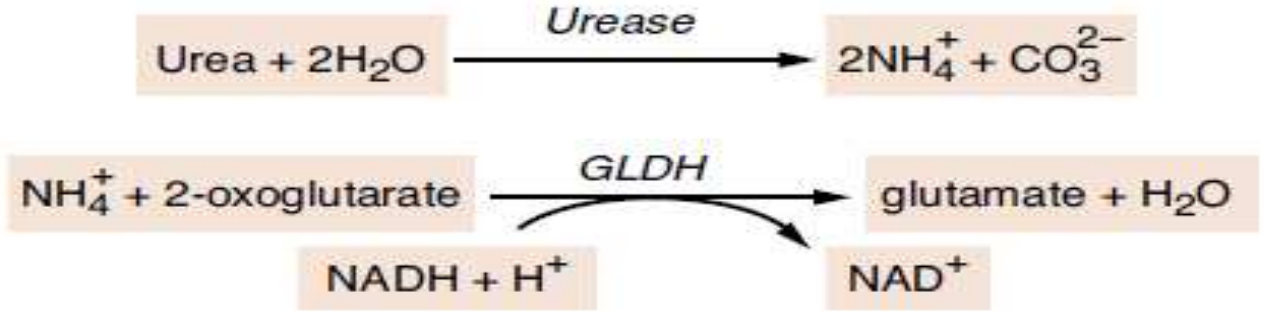
- Hemolysis and lipemic samples.
- Urea is susceptible to bacterial decomposition, so samples (particularly urine) that can not be analyzed within a few hours should be refrigerated.
  - This due to urease producing bacteria such as S.aureus, proteus spp., Klebsiella spp.

Analytical methodology

- The enzyme urease hydrolyzes urea in the sample and the ammonium ion (NH_4) produced in the reaction is quantified.
  - Kinetic approach
  - Endpoint approach

Kinetic approach

- The most common method couples the urease reaction with glutamate dehydrogenase (GLDH) and the rate of disappearance of NADH at 340 nm is measured.



Reagents in use

- Working regent : Urease, GIDH, NADH
- Urea Standard : 80mg/dl

Procedure

Reaction temperature	37^{o}
Working reagent	1000ul
Sample or standard	10ul

✓Mix gently by inversion, insert the cuvette in to the cell holder & start stop watch.

✓Record the initial absorbance exactly after 30 seconds (A1) & exactly after 90 seconds (A2).

✓Calculate the difference between absorbances

Calculation

$$\frac{(A_1 - A_2)_{Sample}}{(A_1 - A_2)_{Standard}} \times C_{Standard} = mg/dL\ urea$$

Endpoint approach

- Ammonium from the urease reaction react with saliciate & hyocholrite to form green dye whose color the intensity is directly proportional to the concentration of urea in the sample. The absorbance is measured at 578nm.

Reagents in use

- RGT 1: Saliciate, Urease
- RGT 2: Hypochlorite
- STD: Urea 80mg/dl

Procedure

	Blank	Standard	Sample
Reagent1	1000ul	1000ul	1000ul
Standard	-----	10ul	-----
Sample	-----	-----	10ul
Mix & incubate at +37^{o} for 5 minutes or 10 minutes at +20-25^{o} c			
Reagent 2	1000ul	1000ul	1000ul
Mix and incubate at +37^{o} for 5 minutes or 10 minutes at +20-25^{o} & read the absorbance against blank			

Calculation

$$C_{test} = \frac{A\ test \times C\ std}{A\ std}$$

Normal values in mg/dl (Urea-N)	Dog	Cat	Cow	Sheep	Goat	Man
	8.8-25.9	15.4-31.2	7.8-24.6	10.3-26.0	12.6-25.8	4.7-23.0

Calculation

- A conversion factor used to correlate the nitrogen content to urea.

✓ Nitrogen gram molecular weight: 14 g/mole
✓ Urea contains 2N: 28 g/mole of urea
✓ Molecular weight of urea: 60 g/mole

60/ 28 = 2.14

Urea N x 2.14 = Urea

Urea x 0.466 = Urea-N