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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment
To determine the urea in blood /serum.

Aim:

To determine the urea in blood /serum.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 53 – 58

Materials Required

Distilled water, blood serum, photometer, pipette and cuvette.

Theory :

Liver converts ammonia (primarily produced by the breakdown of amino acids) into urea. These reactions are used in kinetic enzymatic estimation of urea:

Urea + 2H2O ___urease _____$2NH_4^+ + CO_3^{2-}$

2-oxoglutarate + NH_4^+ + NADH_____glutamate dehydrogenase ___L-glutamate + $NAD^+ + H_2O$

Urea is hydrolysed by urease into ammonia. Ammonia and 2-oxoglutarate are combined by glutamate dehydrogenase to produce glutamate. A decrease in absorbance at 340 nm (Warburg's optical test) is used to photometrically detect the conversion from NADH to NAD in this reaction.

Procedure

1) The photometer should be switched on and allowed to heat for 10 minutes at 37°C.
2) The wavelength should be set at 340 nm and distilled water should be used to make the blanking All of the absorbance that is further described is read using distilled water as a reference.
3) 3 cuvettes are available, i.e., one for blank reaction, one for the standard reaction and one for the sample (blood serum) reaction.
4) **For Blank Reaction**: 002ml of distilled water and 2ml of the reagent (working solution) should be pipetted out into the cuvette. The stop-watch should be pressed at this moment. The solution should be pipetted out inside the cuvette once again up and down to ensure proper mixing. The cuvette should be transferred immediately into the heated photometer. The initial absorbance A should be measured exactly 30 seconds after the working solution has been pipetted, and the second absorbance A_2 should be measured after 1 minute.

A_1 blank	
A_2 blank	

5) 0.02ml of distilled water and 2ml of the reagent (working solution) should be pipetted out into the cuvette. The stop-watch should be pressed at this moment. The solution should be pipetted out inside the cuvette once again up and down to ensure proper mixing The cuvette should be transferred immediately into the heated photometer. The initial absorbance A, should be measured exactly 30 seconds after the working solution has been pipetted, and the second absorbance A, should be measured after 1 minute.

A_1 Standard	
A_2 Standard	

6) 0.02ml of the serum sample and 2ml of reagent (working solution) should be pipetted out. A, and A should be measured as compared to the previous measurements.

A_1 Sample	
A_2 Sample	

7) The differences in absorbance should be calculated.

ΔA blank = A_1blank - A_2blank	
ΔA Standard = A_1 Standard - A_2Standard	
ΔA Sample = A_1 Sample - A_2Sample	

Calculate the Concentration:

Urea (mmol/l) = ΔA Sample - ΔA blank /ΔA Standard - ΔA blank. C standard (15 mmol/l)

Result :

Urea concentration in blood /serum was determined.

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