Particulars of The Experiments Performed

Date	Experiment No.	Subject / Experiment	Page No.	Signature	Re
2/1/2020	1	Estimation of total serum Protein by biuret method		7/2/2020	
7/2/2020	2	Estimation of blood inorganic phosphate by Fiske-Subbarow method		Abas 14/2/2020	
13/3/2020	3	Estimation of serum urea by d-DAM method			

PAGE NO.

DATE 1/2020

PT. NO. 1

ESTIMATION OF TOTAL SERUM PROTEIN BY BIURET METHOD:

INTRODUCTION :—

Serum proteins represent a complex mixture containing a number of compounds which differ in properties and function. The major components of proteins of serum include :— i) Albumin

ii) globulins

iii) Conjugated proteins

such as seromucoid (i.e, glycoprotein or mucoprotein, protein polysaccharide compounds) and lipoproteins.

Fibrinogen is present in plasma and not in serum. Liver is the organ, mainly responsible for formation of plasma albumin and atleast 30% serum globin. Biuret method - is the most commonly used method in clinical practice and is auto analyzer.

PRINCIPLE →

Substances which contain two $-CONH_2$ groups joined together directly or through a single carbon or nitrogen atom and those which contain two or more peptide links, give a blue to purple coloured compound with alkaline copper solution.

SONDHA

The reaction takes its name from the simple substance biuret ($NH_2CONHCONH_2$) gives the same kind of colon with cupric ion. One copper atom complexes with four molecules of biuret, the linkages being to the central nitrogen atom. The amount of colour given varies with different proteins. The method is reliable and reproducible.

REAGENTS :—

Stock Biuret reagent – 45gm of sodium potassium tartarate is dissolved is about 400 ml of 0.2N NaOH (8gm/litre) and 15gm of copper sulphate (finely powdered $CuSO_4, 5H_2O$) is added and stirred continuously until solution is completed 5 gm potassium iodide (KI) is added and made upto 1 litre with (0.2N)NaOH.

Working Biuret reagent – 200 ml of stock reagent is diluted to 1 litre with 0.2 (N) of NaOH containing 5gm of KI/litre
Source of human albumin standard (2 mg/ml) is taken i.e standard BSA is required.

PROCEDURE :—

1. Three test tubes are taken and labeled as Test (T), Standard (S) and Blank (B) respectively.
0.1 ml of goat serum is added in the test tube marked (T)
3 ml of standard BSA solution (2mg/ml) is added in the test tube marked (S).

2.4 ml and 3 ml of distilled water (dH_2O) is added to the test tubes marked (T) and (B) respectively.
3 ml working Biuret Reagent is added to all the three test tubes.
The solutions are mixed and the three test tubes are incubated at 37°C for 10 minutes.
Then cooled at room temperature.
Then optical densities of absorbance are measured against reagent blank at 540 nm.

PROTOCOL –

Reagents↓/Test tubes→	Blank (ml) (B)	Test (T) ml	Standard (S) ml
1) Serum	—	0.1	—
2) Standard	—	—	3
3) Distilled water	3	2.9	—
4) Working Biured reagent	3	3	3

Mixed and incubated at 37°C for 10 minutes. Then cooled at room temperature.

PAGE NO.

Result –

OD of blank (B) = 0

OD of Test (T) = 0.39

OD of standard (S) = 0.13

Calculation –

$$\text{Serum total Protein (mg/dl)} = \frac{RU}{RS} \times \text{Conc. of (S)} \times \frac{100}{V} \times \frac{1}{100}$$

$$= \frac{0.13}{0.39} \times 2 \times \frac{1}{0.1}$$

$$= 6.666 \text{ mg/dl} = 6.6 \text{ mg/dl}$$

Interpretation:–

The normal value of human's serum total protein is 6 gm/dl in the given specimen (goat's serum), the serum total protein value is 6.6 mg/dl, which is much lower than the normal.

2/2/2020

DATE 7/2/2020

EXPT. NO. 2

PAGE NO.

ESTIMATION OF BLOOD INORGANIC PHOSPHATE BY FISKE-SUBBAROW METHOD.

INTRODUCTION :-

The body contains about 17 mod of phosphorus of which 87% is present in bones, and the remainder being found in cells and soft tissues. Phosphorus is a constituent of many important biological compounds, e.g. some proteins, some lipids, nucleic acids and some co-enzymes. It also plays a part in acid-base regulation, particularly by the kidneys.

Phosphorus in blood may be classified as :—

i) Inorganic phosphorus, present as $H_2PO_4^-$ and HPO_4^{2-}.

ii) Organic (or ester) phosphorus, e.g. glycerophosphates, nucleotide phosphate hexose phosphate.

iii) Lipid phosphorus, lecithin, cephalin, sphingomyelin.

iv) A small amount of residual phosphorus.

Red cells are richer in phosphorus than in plasma, mainly because they contains more ester phosphates. When blood is allowed to stand, the inorganic phosphate rises because these organic forms are hydrolysed by enzyme action. The plasma should therefore be promptly separated from the red cells and the proteins precipitated by trichloroacetic acid as quickly as possible.

SONDHA

PAGE NO.

PRINCIPLE:- Proteins in serum are precipitated with trichloroacetic acid. The protein free filtrate containing inorganic phosphate is treated with molybdic acid reagent and phosphomolybdate is formed. This is then reduced to molybdenum blue - a blue solution - by the agent 1-amino, 2-naphthol-4 sulfonic acid (ANSA). The intensity of this blue solution is a measure of inorganic phosphate present.

The reaction can be summarized as follows:—

phosphorus + Ammonium molybdate $\xrightarrow{\text{Acid pH}}$ Ammonium phosphomolybdate.

Ammonium phosphomolybdate + Aminonaphthosulfonic acid $\longrightarrow$ Heteropoly molybdenum.

REAGENTS:-

- 10% Trichloroacetic acid (TCA)
- Sulphuric acid (10N) - Add 450 ml of concentrated sulphuric acid. Add slowly whilst cooling to 1300 ml of distilled water. Dilute some of this 1 in 10 and titrate with 1N Sodium hydroxide and make any necessary adjustment in the original.
- Molybdic acid reagent (2.5% ammonium molybdate in 3N H_2SO_4): Dissolve 25g ammonium molybdate in about 200 ml water and transfer to one litre flask containing 300 ml of 10N H_2SO_4. Dilute to 1 litre with water stable indefinitely.

DATE

EXPT. NO.

PAGE NO.

⊙ Aminonaphthol sulfonic acid (ANSA) reagent :- Add 0·5g of 1 amino-2-napthol-4-sulfonic acid to 195 ml of 15% sodium bisulphite and 5ml of 20% sodium sulfite in a conical flask. shake until it dissolves. Warm a little and then filter. The reagent should be prepared fresh as it is stable for 4 weeks only. Store in brown bottle in refrigerator.

⊙ Standard phosphate solution :- Dissolve 0·351 g of pure dry potassium dihydrogen phosphate in one litre flask. Add 10 ml of 10N H_2SO_4 add dilute to the mark with water. The standard is stable indefinitely. 5ml of this contains 0·4 mg phosphorus.

⊙ Working standard :- 1:10 so that the 5ml contains 0·04mg of phosphorus.

PROCEDURE :-

i) Pipette 2ml serum / blood into a test tube and add 8 ml trichloroacetic acid with constant shaking.

ii) Allow to stand for 5 minute, and then filter

iii) Collect the filtrate.

iv) label 3 test tubes as B. S and T and then add 5ml TCA, 05 ml of working standard and 5ml of filtrate to the test tubes respectively.

SONDHA

v) Now add the molybdate solution (1ml) to all the 3 test tubes

vi) Add ·4ml of ANSA to the 3 test tubes.

vii) Make the volume upto 10ml by adding distilled water.

DATE

EXPT. NO.

PAGE NO.

viii) Wait of 5 minutes.

Reagents	Test	Standard	Blank
1) filtrate	5ml	—	—
2) Standard Phosphate	—	5ml	—
3) 10 %. TCA	—	—	5ml
4) Molybdate II	1ml	1ml	1ml
5) ANSA	0.4ml	0.4ml	0.4ml

Volume make upto 10ml
wait for 5 minutes
OD measure by colorimeter at 660 - 720 nm

RESULT:—

Reading of blank = 0
Reading of unknown = 1.03
Reading of standard - 2.11

CALCULATION:—

For photocholorimetric measurement = (Ref. Hawk's Practical Physio-logical chemistry)

$\frac{\text{Density of unknown}}{\text{Density of standard}} \times 0.04 \times 100$ = mg. inorganic phosphate per 100ml whole blood plasma or serum.

SONDHA

DATE

EXPT. NO.

PAGE NO.

$= \frac{1.03}{2.11} \times 0.04 \times 100$

= 1.95 mg of inorganic phosphate /100ml whole blood.

INTERPRETATION:-

Normal serum inorganic phosphate in adults is 3.0-4.5mg /dl and in children is 4.0-6.0 mg/dl. Here, the serum (goat) inorganic phosphate is 1.95 mg/dl.

ADas
14/2/2020.

DATE 13/3/2020

PAGE NO.

EXPT. NO. 3

ESTIMATION OF SERUM UREA BY DAM METHOD

INTRODUCTION—

Urea is a main end product of protein catabolism. Deamination of amino acid takes place in the liver which is also the site of urea cycle. Whereby ammonia released is converted into urea and is excreted through urine. It represents about 45-50% of non-protein nitrogen of the blood and about 80-89% of the total urinary nitrogen of the blood and about nitrogen excretion. Urea N_2 varies directly with protein intake and inversely with the rate of excretion of urea.

PRINCIPLE:—

Urea may be measured without conversion to ammonium by making use of ability to react with Diacetyl Monoxime (DAM)

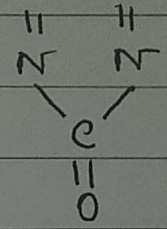
Under acetic condition when urea is heated with compounds containing to adjacent carbonyl groups such as—

Diacetyl [$CH_3COCOCH_3$] coloured products are formed. Diacetyl mono [$CH_3COC=NOHCH_3$] has usually being used because of greater stability. On heating, it decomposer to give hydroxylamine and diacetyl, which then condencer with urea. to give Diazine.

SONDHA

$CH_3CO = NOHCH_3 + H_2O \longrightarrow CH_3COCOCH_3 + NH_2OH$

$CH_3COCOCH_3 + CO(NH_2)_2 \longrightarrow$



(Diargine)

REAGENTS:

- **Reagent-A:** 1 gm of $FeCl_3$ dissolved in 4 ml of distilled water. This mixture was transferred to a graduated cylinder and 20 ml of orthophosphoric acid (85%) was slowly mixed by stirring. The volume was made upto 50 ml with water. This reagent was kept in brown bottle at 4°C.

- **Reagent-B:** 20 ml of conc. H_2SO_4 (98%) was added to 80 ml of water in a 200 ml flask slowly with stirring and keeping it in a ice bath.

- **Reagent-C:** Diacetyl monoxime was prepared at a conc. of 2 gm/100 ml of water. Filtered and kept in brown bottle at 4°C.

- **Reagent-D:** Thiosamine carboxide was prepared at a conc. of 5 gm/100 ml of distilled water.

PAGE NO.

DATE

EXPT. NO.

- Acid Reagent: 0.1 ml of reagent A added to 199.9 ml of reagent - B and kept it in a brown bottle of 4°C.

- Colour reagent: 67 ml of reagent C and reagent D was added and mixed upto 1000 ml with distilled water. The reagent was kept in a brown bottle at 4°C.

- Stock urea standard: This standard was prepared at a conc. of 100 mg urea / 100 ml of distilled water.

- Working standard: 1 ml of stock urea standard was diluted to 100 ml with distilled water.

PROCEDURE:

- The serum was diluted with water at a ratio of 1:100.
- The test - tubes were arranged for the experiment according to
- The following table -

Reagents ↓ / Test tubes →	Test (T)	Standard (S)	Blank (B)
i) Serum	1 ml	–	–
ii) Standard	–	1 ml	–
iii) Distilled water	1 ml	1 ml	2 ml
iv) Colour reagent	2 ml	2 ml	2 ml
v) Acid reagent	2 ml	2 ml	2 ml

SONDHA

PAGE NO.

DATE

EXPT. NO.

⊙ Each test tube was mixed thoroughly and the tubes were placed in boiling water bath for 20 minutes.

⊙ Then the test tubes were cooled and the OD was measured at 520 nm.

RESULTS:

Reading of blank - 0.00

Reading of Test - ~~0.24~~ 1.14

Reading of standard - 0.14

CALCULATION –

$$\text{Blood urea} = \frac{\text{Optical density of test}}{\text{optical density of Standard}} \times \frac{\text{Conc. of standard}}{\text{effective volume of serum}} \times 100$$

$$= \frac{1.14}{0.24} \times \frac{0.01}{0.01} \times 100$$

$$= 4.75 \times 100$$

$$=$$

SONDHA

Date	Experiment No.	Subject / Experiment	Page No.	Signature
7/2/2020	1	Determination of hand reaction time.		

Page No. :	1
Date :	7/2/2020
Expt. No. :	1

DETERMINATION OF HAND REACTION TIME

PRINCIPLE :— Reaction time is measured between the presentation of stimulus out the response of the object. Measurement of reaction time is based on the basis of constant accumulation of free falling bodies and consists of a which scale to read in time as compared from following formula —

$$\text{Reaction time (in sec)} = \sqrt{\frac{2 \times \text{distance stick fall}}{\text{Acceleration due to gravity}}}$$

PROCEDURE :— The subject sits with the forearm and hand resting comfatably in the table on desk. The tifas of the thumbus and the index finger are held in a ready to pinch position about 3' to 4" beyond the edge of the thumb or table. The upper edge of the thumb and index finger should be in a horizontal position. The stick in held near the top it hangs between the subjects hand and index finger. The base line on the stick ~~is held~~ should be oven with the upper surface of the subjects thumb to reacts with the catching the stick when it is released. The subject should not took at the testeri hand or move the hand up or down while attempting to catching the fallen stick. 20 trials are given each drop in preceed by a reparately command on ready. The process in 2 hands

Teacher's Signature ....

individually.

SCORING:- When the subject catches the sticks, the reading is taken just above the upper edge of the thumb. The slowest and fastest 5 reading are discovered and middle to 10 are recorded as the scores. Then the mean of the reading is calculated.

RESULT:-

Name of the subject - Smiriti Bisal

Age - 20 years Sex - Female

Height - 5.4ft Weight - 56 Kg.

	Right hand			Left hand.			
Obs no	difference (cm)	Middle 10 reading (cm)	Mean (cm)	Obs no	difference (cm)	Middle 10 reading (cm)	Mean (cm)
1	20	20		1	(22)		
2	(3)			2	(10.5)		
3	(9)			3	14	14	
4	(23)			4	18	18	
5	16	16		5	20.9	20.9	
6	(8)			6	(29)		
7	18	18		7	16.8	16.8	
8	(26.3)			8	11.6	11.6	

Teacher's Signature .....

Page No.	:
Date	:
Expt. No.	:

Right hand				left hand			
Obs no	difference (cm)	Middle 10 reading (cm)	Mean (cm)	obs no	difference (cm)	Middle 10 reading (cm)	Mean (cm)
9	(5.8)			9	19	19	
10	17.3	17.3		10	(24)		
11	17.2	17.2		11	17.7	17.7	
12	(29.2)			12	(24.9)		
13	18.3	18.3		13	(9)		
14	13.9	13.9	8.14	14	(3.9)		16.08
15	16.8	16.8		15	(26)		
16	(11.9)			16	12.8	12.8	
17	18.9	18.9		17	15	15	
18	(28)			18	15	15	
19	(21)			19	(9)		
20	20	20		20	(7.5)		

Reaction time of Right hand = $\sqrt{\frac{2 \times \text{distance stick fall}}{\text{Acceleration due to gravity}}}$

$= \sqrt{\frac{2 \times 8.14}{981}}$

$= 0.128$ cm

Teacher's Signature .....

Page No. :
Date :
Expt. No. :

Reaction time of left hand = $\sqrt{\frac{2 \times \text{distance stick fall}}{\text{Acceleration due to gravity}}}$

$= \sqrt{\frac{2 \times 16.08}{981}}$

$= 0.181$ cm.

DISCUSSION :- For University student age between 22 - 24 hours the avarage of mean value is arround 0.125 sec with a range of 0.13 to 0.22 sec. For children the average in 0.25 sec. therefore here in this experiment the reaction time of the subject is 0.128 and 0.181 in right and left hand respectively which is with in the range of normal reaction time score. The reaction time of right hand is lesser than the left hand.

INTERPETATION :- Reaction time depends upon the different factor. Some factor reduce this and score increase this. Reaction time is also influenced by stimulus intensity. Increased stimulus intensity. Increased stimulus intensity reduces reaction time. Hand reaction time in neceasary for any work. Noise and vibration increases the reaction time to bring accuracy the determination of reaction time should be done at calm condition.

Teacher's Signature .....