15BCU611 PRACTICAL- VI 5H-3C
CLINICAL BIOCHEMISTRY

BLOOD ANALYSIS

1. Determination of blood sugar by O-Toluidine method.
2. Determination of urea DAM-TSC method.
3. Determination of phosphorus by Fiske-Subbarow method.
4. Determination of alkaline phosphatase in serum.
5. Determination of acid phosphatase in serum.
6. Determination of cholesterol in serum by Zak's method.
7. Determination of total proteins by Lowry's method and Biuret method.

URINE ANALYSIS

8. Determination of creatinine by picric acid method.
9. Determination of urea DAM-TSC method.
10. Determination of uric acid by Caraway's method.
11. Determination of calcium by permanganate method.
12. Determination of phosphorus by Fiske-Subbarow method.

KIT METHOD (Group experiments)

13. Estimation of triglycerides in serum
14. Estimation of creatinine in serum
15. Estimation of haemoglobin in serum
16. Extraction of lipids from liver and estimation of cholesterol

REFERENCES

Jayaraman.J.2007. Laboratory Manual in Biochemistry, New Age International Publishers, New Delhi.

Patel.H .2003.A Manual of Medical Laboratory Technology, Navaneet Prakashan limit, Bombay.

Sadasivam.S, and A. Manickam .2009.Biochemical Methods, New Age International Publishers, New Delhi.

Singh.S.P.2009. Practical Manual of Biochemistry, CBS Publishers, New Delhi.

KARPAGAM ACADEMY OF HIGHER EDUCATION
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Coimbatore – 641 021.

LESSON PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Dr.S.PRIYANGA
SUBJECT NAME: CLINICAL BIOCHEMISTRY
SUB.CODE: 15BCU611 SEMESTER: VI CLASS: III B.Sc (BC)

S.NO	NAME OF THE EXPERIMENT	SUPPORT MATERIALS
BLOOD ANALYSIS		
1.	Determination of blood sugar by O-Toluidine method.	T1: 8-9
2.	Determination of urea DAM-TSC method.	T2: 158-159
3.	Determination of phosphorus by Fiske-Subbarow method	T2: 445-450
4.	Determination of alkaline phosphatase in serum.	T1:121-122
5.	Determination of acid phosphatase in serum.	T1:122-123
6.	Determination of cholesterol in serum by Zak's method.	T3: 179-180
7.	Determination of total proteins by Lowry's method and Biuret method.	T1:56-57
URINE ANALYSIS		
8.	Determination of creatinine by picric acid method	T3: 182-184
9.	Determination of urea DAM-TSC method	T2: 158-160
10.	Determination of uric acid by Caraway's method	T3: 185-186
11.	Determination of calcium by permanganate method	T2: 442-443
12.	Determination of phosphorus by Fiske-Subbarow method	T2: 450-452
KIT METHOD (Group experiments)		
13.	Estimation of triglycerides in serum Estimation of creatinine in serum	
14.	Estimation of haemoglobin in serum Extraction of lipids from liver and estimation of cholesterol	

Text Books

1. Sadasivam S and Manickam A. 2009. Biochemical methods, New age International publishers, New Delhi.

2. Harold Varley, 2005. Practical Clinical Biochemistry, 4th edition, CBS publishers Pvt Ltd. New Delhi.

3. Rajan S and Selvi Christy R, 2010. Experimental procedures in life sciences, 1st edition, Classik printers, Chennai.

SYLLABUS

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KIT METHOD (Group experiments)

13. Estimation of triglycerides in serum
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16. Extraction of lipids from liver and estimation of cholesterol

Determination of Glucose in serum

Ortho Toludine Method

Aim

To estimate the amount of glucose present in the given serum sample.

Principle

Glucose reacts with orthotoluidine in glacial acetic acid in the presence of heat to yield blue green N-glucocyl amine and that can be read at 630nm in a colorimeter.

Reagents

1. Glucose stock standard solution:

200mg of glucose is dissolved in 100ml of distilled water

2. Glucose working standard solution:

10ml of stock standard is diluted to 100 ml of distilled water

3. Orthotoludine reagent:

Transfer 15g of thiourea to a 1litre erylenmayer flask and 910ml of glacial acetic acid, 90ml of orthotoludine. Mixed well until the thiourea was dissolved. Stored at room temperature in a brown bottle.

Procedure

0.2ml of serum was mixed with 1.8ml of 10% TCA. Centrifuge and 1.0ml of supernatant also taken 0.5, 1.0, 1.5, 2.0, 2.5ml of standard glucose solution corresponding to the values 50, 100, 150, 200, 250 µg respectively and made upto 2.5ml of orthotoludine reagent and mixed thoroughly and keep the tubes in a boiling water bath for 10 minutes.

Remove the tubes and brought to room temperature by putting them in cold water bath for 10 minutes. Determine the optical density of solution in various standards and solution using the red filter using blank reagent read at 630nm.

Result

The amount of glucose present in the given serum is ---------- mg/dl.

Determination of Urea in serum and urine

DAM TSC Method

Aim

To estimate the amount of urea present in urine and blood

Principle

Urea directly reacts with diacetylmonoxime in the presence of thiosemicarbazide to form a red coloured product that is measured colorimetrically at 540nm.

Reagents

1. Diacetyl monoxime:

1.56g of diacetylmonoxime in 250ml of distilled water

2. Thiosemicarbazie:

41mg of thiosemicarbazied in 250 ml of distilled water (stored in brown bottle)

3. Ferric chloride reagent:

324mg ferric chloride in 10ml of 50% orthophosphoric acid (Stored in brown bottle).

4. 20% sulphuric acid
5. Stock standard urea solution:

Dissolved 100mg of urea in 100ml of distilled water. This solution is prepared in saturation solution of benzoic acid for long use. 1.0ml of this solution contains 1mg of urea

6. Working standard urea solution:

2.0ml of stock standard was diluted to 100ml with distilled water. 1.0ml of their solution contains 20 µg of urea

Procedure

Take 1.8ml of trichloroacetic acid and 0.2ml of blood centrifuge for 10 minutes. 0.5ml of supernatant was taken for experiment into a series of test tubes take 0.5, 1.0, 1.5, 2.0 and 2.5ml of working standard urea solution. Corresponding to the values of 10, 20, 30, 40 and 50 µg respectively. The volume was made-up to 3.0ml with distilled water n all the test tubes. Added 1.0ml of diacetylmonoxime, 1.0 m of thiosemicarbazide and 3.0ml of acid reagent and heated vigorously in boiling water for 20 minutes. Along with this a blank was also conducted. Remove the tubes and cool. Reading were taken against the reagent blank at 540nm in a colorimeter.

A standard graph was drawn by plotting colorimetric reading on y axis and concentration of urea on x axis.

Normal Values

Blood – 19-30 mg/dl

Urine

Sample preparation:

1.0ml of urine was made upto 100ml with distilled water. 1.5ml of diluted urine was taken for the experiment.

Result

The amount of urea present in the given serum sample is ---------- mg/dl

The amount of urea present in the given urine sample is ---------- mg/dl

Determination of Phosphorus in serum and urine sample

Fiske and Subbarow's Method

Aim

To estimate the amount of phosphorus present in the given sample.

Principle

Phosphorus reacts with molybdic acid to form phosphor molybdic acid. On treatment with 1,2,4 amino napthol sulphonic acid (ANSA), phosphor molybdic acid is reduced to produce a deep colour (molybdenum blue) which is a mixture of lower acids of molybdenum. The blue colour is measured spectropotometrically at 660nm.

Reagents

1. **2.5% ammonium molybdate:**

Dissolve 25 g of ammonium molybdate in 200ml water and transferred to 1 L flask containing 300 ml of 10 N H_2So_4 and diluted to 1L with water.

2. **Amino Napthol Sulphonicacid Reagent (ANSA)**

195ml of 15% sodium bisulphate is taken in a glass stoppered cylinder and 0.5 g of 1,2,4 aminonaptholsulphonic acid is added to it follwed by 5.0ml of 20% sodium sulphite. It is stoppered and shaken until the powder is dissolved. If the solution is not complete, added more sodium sulphite, 1.0ml at a time with shaking. The solution is then transferred to a brown glass bottle and stored in the cold. This solution is usable for 4 weeks.

3. **Stock cholesterol reagent**

Accurately weighed 35.1 mg of mono potassium hydrogen phosphate (KH_2PO_4), dissolved in water and added 1.0ml of 10N sulphuric acid and made up to 100 ml with water. 1.0ml of solution contains 80 µg of phosphorus.

4. **Working standard:**

10 ml of the stock standard phosphorus solution was diluted to 100 ml with distilled water. 1.0ml of the working standard solution contains 8 µg of phosphorus.

Procedure

Into a series of test tubes pipetted out 1.0 – 5.0 ml of working standard solution corresponding to µg values 8-40. 1.0ml of the unknown solution and 0.5ml of the sample solution was taken in separate test tubes. The volume in all the tubes was made up to 8.6 ml with distilled water. Set up a blank with 8.6 ml of distilled water. Added 1.0ml of 2.5% ammonium molybdate and 0.4ml of ANSA to all the tubes. Mixed well and allowed to stand for 10 minutes.

The blue colour developed was read at 66onm in a spectrophotometer. A standard graph was drawn by plotting the concentration on x axis and the optical density on y axis. From this, the concentration of phosphorus in the given sample solution was calculated.

Result

1. The amount of phosphorus present in 100 ml of the given serum sample was found to be ---------- mg.
2. The amount of phosphorus present in 100 ml of the given urine sample was found to be ---------- mg.

Determination of Cholesterol in serum

ZAK's Method

Aim

To estimate the amount of cholesterol present in the given sample of blood.

Principle

Cholesterol reacts with ferricchloride in the presence of concentrated sulphuric acid to give a pink colouor. The intensity of the colour developed is directly proportional to the amount of cholesterol and it is read at 540nm in colorimeter.

Reagents

1. Ferric chloride reagent:

840mg of pure dry ferric chloride weighed and dissolve in 100 ml of glacial acetic acid.

2. Ferric chloride diluting agent

8.5 ml stock ferric chloride is diluted to 100ml with glacial acetic acid in a 100ml standard flask.

3. Stock cholesterol reagent

100mg of pure dry cholesterol was dissolve in glacial acetic acid in 100ml of standard flask.

4. Working standard:

10ml of stock standard was placed in 100ml of standard flask. Added 0.85ml of ferric chloride stock reagent and madeup to the mark with glacial acetic acid. 1.0ml of the solution contains 100 µg of cholesterol.

5. Ferric chloride precipitation reagent:

10ml of flask stock ferric chloride was taken in a 100ml standard flask and made upto the mark with the glacial acetic acid.

Procedure

0.5-2.5ml working standard cholesterol solution was pipetted out into a clean dry tube. Total volume of the each test-tube was made upto 5.oml with ferric chloride. Mixed well the test tubes were kept in cold water.

To each tube added 4.0ml of concentrated sulphuric drop by drop the solution was mixed well and blank was prepare simultaneously by taking 5.0ml of diluting agent and 4.0ml of concentrated sulphuric acid after 30 minutes. The intensity of colour development was read at 540nm against the reagent blank.

Treatment of serum sample

0.1ml serum was added with 4.9ml of ferric chloride precipitating agent and mixed well. Allow standing for a while and centrifuged. Transfer 2.5ml of clear supernatant into a dry test tube and added 2.5ml of ferric chloride diluting agent and mixed well. The tubes were kept in cold water and to each tube added 4.0ml of concentrated H_2So_4 drop by drop the solution were

mixed well. The tubes are simultaneously prepared by taking 5.oml of diluting reagent and 4.oml of concentrated sulphuric acid.

A standard graph was drawn by plotting concentration on x axis and colorimetric reading on y axis from this the concentrating cholesterol was calculated.

Normal Values

150-250mg/dl

Result

The amount of cholesterol present in the given serum sample is ---------- mg/dl

Determination of Calcium in serum and urine

Permanganate Method

Aim

To estimate the amount of calcium in the given sample of urine and serum

Principle

Calcium in urine and serum is precipitated as calcium oxalate with ammonium oxalate (Magnesium is not precipitated) as the conditions are selected to increase the solubility of magnesium oxalate.

The precipitate is washed with dilute ammonia to remove the excess ammonia oxalate and then dissolved in normal sulphuric acid. The oxalic acid formed in titrated with standard permanganate solution. The endpoint of titration is indicated by the formation of a pink colour stable for at least 30 seconds.

Reagents

1. 4% Ammonium oxalate solution
2. 2% Ammonia (v/v)

Diluted 2.0ml of ammonia (specific gravity 0.88) to 100ml with water.

3. 0.01N Potassium permanganate solution:

Prepared freshly before use by diluting the stock potassium permanganate (0.1N)

4. Approximate Normal sulphuric acid

Procedure

In to a each of two centrifuge tubes, pipetted out 2.0ml of urine or serum and 1.0ml of ammonium oxalate. Mixed the tubes and allowed to stand overnight at room temperature. Centrifuged for 10minutes at 2000rpm and carefully draied the supernatant. Inverted the tubes and allowed to drain on a pad of filter paper for 3 minutes. Added 3.0ml of 2% ammonia down the sides of the tubes. Mixed the precipitate, centrifuged and decant the solution. This process was repeated until the precipi8tate was washed completely and supernatant gave no precipitate with calcium chloride.

Pipetted out 2.0ml of normal sulphuric acid, rotating the tubes to wash down then placed the tubes in water bath for 70-80^{o} C for five minutes to dissolve the precipitate. Removed and titrated the contents hot with 0.01N potassium permanganate solution taken in a burette till a pale pink colour was got which persists for about a minute. Repeat the titration with a duplicate tube.

Performed a blank titration with 2.0ml of normal sulphuric acid, kept in water bath for 5 minutes and titrated with permanganate to a pink colour. The difference between the two tubes titre values gives the volume of 0.01N potassium permanganate required to titrate cacium-oxalate.

Result

1. The amount of calcium present in 100ml of urine sample ---------- mg
2. The amount of calcium present in 100ml of serum sample ---------- mg of calcium.

Determination of Protein

Lowry *et al.*, 1957

Aim

To estimate the amount of protein present in the given serum sample.

Principle

The blue color developed by the reduction of the phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein

plus the color developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured at 660nm.

Reagents

1. 2% Sodium carbonate in 0.1N NaOH (Reagent A)
2. 0.5% Copper sulphate in 1% potassium sodium tartarate (Reagent B)
3. Alkaline copper reagent:

Mixed 50ml of A and 0.1ml of B prior to use.

4. Folin-Ciocalteau reagent:

Mixed 1 part of reagent with 2 parts of water.

5. Stock standard:

Weighed 50mg of bovine serum albumin and made up to 50ml in a standard flask with saline.

6. Working standard:

Diluted 10ml of the stock with 50ml of distilled water. 1.0ml of this solution contains 200µg of protein.

Procedure

Pipetted out 0.2 to 1.0ml working standard solution, 0.1ml of the sample was taken. The volume in all the tubes was made up to 1.0ml with distilled water. Added 5.0ml of alkaline copper reagent to each tube. Mixed well and allowed to stand for 15 minutes. Then added 0.5ml of Folin-Ciocalteau reagent. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes, the blue color developed was read at 660nm.

A standard graph was drawn by plotting the concentration on x axis and the optical density on y axis. From this, the concentration of protein in the given sample solution was calculated.

Result

The amount of protein present in the given solution was found to be ---------- g/dl

Determination of acid phosphatase (ACP)

King, 1965

Aim

To estimate the amount of acid phosphatase present in the given sample.

Principle

The method used was that of King and Armstrong in which disodium phenylphosphate is hydrolyzed with the liberation of phenol and inorganic phosphate. The liberated phenol is measured at 700nm with Folin-Ciocalteau reagent.

Reagents

1. Citrate buffer: 0.1M, pH 5.

   A: Citric acid (21.01g in 100ml)

   B: Sodium citrate (29.41g in 100ml)

2. Disodium phenyl phosphate, 100mmol/L:

   Dissolved 2.18g of disodium phenylphosphate in distilled water and heated to boil, cooled and made to a litre. Added 1.0ml of chloroform and stored in the refrigerator.

3. Buffered substrate:

   Prepared by mixing equal volume of the above two solutions. This has a pH of 5.0.

4. Folin-Ciocalteau reagent:

   Prepared by mixing one volume of reagent and two volumes of water. Sodium carbonate solution, 15%: Dissolved 15g of anhydrous sodium carbonate in 100ml water.

5. Standard phenol solution, 1g/L:

   Dissolved 1g pure crystalline phenol in 100mmol/L HCl and made to a litre with acid.

6. Working standard solution:

   Diluted 10ml of stock standard to 100ml with water. This contains 100µg of phenol/ml.

Procedure

Pipetted out 4.0ml of buffered substrate into a test tube and incubated at 37˚C for 5 minutes. Added 0.2ml of sample and incubated further for exact 60 minutes. Removed and immediately added 1.8ml of diluted phenol reagent. At the same time, set up a control containing 4.0ml buffered substrate and 0.2ml of sample to which 1.8ml of phenol reagent was added immediately. Mixed and centrifuged. To 4.0ml of supernatant added 2.0ml of 15% sodium carbonate. Took 4.0 ml of working standard solution and for blank taken 3.2 ml water and 0.8ml of phenol reagent. Then added 2.0 ml of sodium carbonate. Incubated all the tubes at 37˚C for 5 minutes. Read the color developed at 700nm.

A standard graph was drawn by plotting the concentration on x axis and the optical density on y axis. From this, the concentration of acid phosphatase in the given sample solution was calculated.

Result

The activity of serum acid phosphatase was found to be ---------- μmoles of phenol liberated per litre.

Determination of alkaline phosphatase (ALP)

King and Armstrong, 1934

Aim

To estimate the amount of phosphorus present in the given sample solution

Principle

The method used was that of King and Armstrong in which disodium phenylphosphate is hydrolyzed with the liberation of phenol and inorganic phosphate. The liberated phenol is measured at 700nm with Folin-Ciocalteau reagent.

Reagents

1. Sodium carbonate-Sodium bicarbonate buffer, 100mmol/L:

Dissolved 6.36g anhydrous sodium carbonate and 3.36g sodium bicarbonate in water and made to a litre.

2. Disodium phenyl phosphate, 100mmol/L:

Dissolved 2.18g of disodium phenylphosphate in distilled water and heated to boil, cooled and made to a litre. Added 1.0ml of chloroform and stored in the refrigerator.

3. Buffered substrate:

Prepared by mixing equal volume of the above two solution. This has a pH of 10.

4. Folin-Ciocalteau reagent:

Prepared by mixing one volume of reagent and two volumes of water.

5. Sodium carbonate solution, 15%:

Dissolved 15g of anhydrous sodium carbonate in 100ml water.

6. Standard phenol solution, 1g/L:

Dissolved 1g pure crystalline phenol in 100mmol/L HCl and made to a litre with acid.

7. Working standard solution:

Added 100ml diluted phenol reagent to 5.0ml of stock standard and diluted to 500ml with water. This contains 10μg of phenol/ml.

Procedure

Pipetted out 4.0ml of buffered substrate into a test tube and incubated at 37˚C for 5 minutes. Added 0.2ml of sample and incubated further for exact 15 minutes. Removed and immediately added 1.8ml of diluted phenol reagent. At the same time, set up a control containing 4.0ml buffered substrate and 0.2ml of sample to which 1.8ml of phenol reagent was added immediately. Mixed well and centrifuged. To 4.0ml of supernatant added 2.0ml of 15% sodium carbonate. Took 4.0ml of working standard solution and for blank taken 3.2ml water and 0.8ml of phenol reagent. Then added 2.0ml of sodium carbonate. Incubated all the tubes at 37˚C for 15 minutes. Read the color developed at 700nm.

A standard graph was drawn by plotting the concentration on x axis and the optical density on y axis. From this, the concentration of alkaline phosphatase in the given sample solution was calculated.

Result

The activity of serum alkaline phosphatase was found to be ---------- μmoles of phenol liberated per litre

Estimation of Creatinine

Owen *et al.*, 1954

Aim

To estimate the amount of creatinine present in the given sample.

Principle

Creatinine forms a coloured complex with picrate in alkaline medium. The rate of formation of the complex is measured at 540 nm.

Reagents

1. **Picric acid:**

8.02g picric acid/litre

2. **Sodium hydroxide:**

12.8g sodium hydroxide/litre

3. **Reagent mixture:**

Mixed one part by volume of diluted NaoH with one part by volume of picric acid at least 30minutes before the assay.

4. **Stock standard:**

Dissolved 100mg of creatinine in 100 ml of distilled water.

5. **Working standard:**

2.0ml stock diluted to 100ml with distilled water. This contained 20 µg/ml of creatinine/ml.

Procedure

Pipetted out 0.2ml of serum and 2.0ml of the reagent mixture into a cuvette. Simultaneously, a blank were set up with the reagent mixture and distilled water. Mixed well and the change in absorbance were measured after 30 sec, which were taken as A_1 and exactly after 2 min, the absorbance were read as A_2 at 490nm. A set of standards were also treated in the same manner. A_2-A_1 gives the change in absorbance, which was the measure of the creatinine present in the sample. A standard graph was drawn by plotting the concentration on x axis and the optical density on y axis. From this, the concentration of creatinine in the given sample solution was calculated.

Result

The activity of serum creatinine was found to be --------- mg/dl.

Determination of Uric Acid

Caraway Method

Aim

To estimate the amount of uric acid present in the given sample.

Principle

Uric acid reduces sodium phosphotungstate in the given alkaline medium to give a blue colour, which is measured colorimetrically using a red filter at 640nm.

Reagents

1. 14% Sodium carbonate solution
2. Uric acid Reagent:

Dissolved 5g of sodium tungstate in 400 ml of distilled water. Added 40ml of 84% phosphoric acid and refluxed gently for 2 hours. Cooled, transferred to 50ml standard flask and made upto the mark with distilled water. Stored the reagent in a brown bottle. Dluted 1-10 ml before use.

3. Stock standard:

Weighed about 100mg of uric acid in a small beaker. Also weighed 60mg of lithium carnbonate into the beaker. Added 15-20ml of water heated the solution to about 60^{o}C and poured into 100ml standard flask and made upto the mark with distilled water.

4. Working standard:

Diluted 2ml of stock standard to 100ml with water. 1ml of the working standard containing 20µg of uric acid.

Procedure

In to different test tubes pipetted out 0.5, 1.0, 1.5, 2.0, 2.5ml of working standard corresponding to the µg values 10-50 respecively and made upto 3ml with distilled water. The given unknown solution was made upto 100ml with distilled water. From this 20ml was taken for the experiment. This was made upto 3ml with distilled water.

To all the tubes added 1ml of uric acid reagent follower by 1ml of 14% sodium carbonate solution and let it stood for 15minutes and the blue colour developed was read in the colorimeter at 640nm against a reagent blank.

A standard graph was drawn by plotting the concentration of uric acid on the x-axis and colorimeter reading on y-axis. From this experiment the concentration of uric acid present in the unknown solution was calculated.

Result

The amount of uric acid present in 100ml of the given sample is ---------- mg/dl.

KIT METHOD (Group Experiments)

Estimation of Triglycerides (Trinder, 1969)

Aim

To estimate the amount of triglycerides present in the given sample.

Principle

Glycerol released from hydrolysis of triglycerides by lipoprotein lipase is converted by glycerol kinase into glycerol-3-phosphate which was again oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxide, hydrogen peroxide oxidizes phenolic chromogen to a red colored compound.

Reagents

- Reagent 1: Triglycerides enzyme reagent
- Reagent 2: Triglycerides standard 200 mg/dl

Procedure

Addition sequence	Blank	Standard	Test
Enzyme reagent	1 ml	1 ml	1 ml

Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed well and incubated for 10 minutes at 37ºC. Final color developed was stable for 30 minutes and the absorbance of standard and sample against the reagent blank were measured at 505 nm.

Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 200$$

Result

The amount of triglyceride was found to be _____ mg/dl.

Creatinine (Tanganelli *et al*., 1982)

Aim

To estimate the amount of creatinine present in the given sample.

Principle

Creatinine reacts with picric acid to produce a colored compound creatinine alkaline picrate. The change in absorbance was proportional to the creatinine concentration.

Reagents

- Creatinine base reagent (R1)

Sodium hydroxide: 300 mM/L

Sodium phosphate: 25 mM/L

- Creatinine dye reagent (R2)

Picric acid: 8.73 mM/L

- Creatinine standard (2 mg/dL)
- Working reagent: Mix 1 volume of R1 with 1 volume of R2

Procedure

Contents added	Standard	Sample
Working reagent	1000µl	1000 µl
Standard	100 µl	-
Sample	-	100 µl
Mixed well and read the optical density at 492nm (T1) 60 seconds after the addition of samples and standard. Exactly 60 seconds after the first reading the second reading (T2) was taken		

Calculation

$$\text{Creatinine concentration (mg/dl)} = \frac{\text{(T1-T2) of sample}}{\text{(T1-T2) of standard}} \times 2$$

Result

The amount of creatinine was found to be _____ mg/dl.

Estimation of hemoglobin (Drabkin and Austin, 1932)

Aim

To estimate the amount of hemoglobin present in the given sample.

Principle

In alkaline medium, hemoglobin and its derivatives were oxidized in the presence of potassium ferricyanide and get converted to methemoglobin, which then reacts with potassium cyanide to form purple red colored cyanmethemoglobin complex, the intensity of which was measured at 546 nm.

Reagents

- Drabkin's solution
- Cyanmethemoglobin standard: 65 mg/dl

Procedure

0.02 ml of blood and 5.0 ml of Drabkin's solution was pipetted out into a test tube. Simultaneously, a blank was set up with Drabkin's solution and distilled water. Mixed well the above tubes and allowed to stand at room temperature for 5 minutes. The absorbance of test was

measured at 546 nm. The absorbance of cyanmethemoglobin standard was taken directly without adding working reagent against blank at 546 nm.

Result

The results were expressed as g/dl in blood.

Estimation of Cholesterol (Richmond, 1973)

Aim

To estimate the amount of cholesterol present in the given sample.

Principle

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxide to form a red colored quinoneimine dye complex. Intensity of the color formed was directly propotional to the amount of cholesterol present in the sample.

Reagents

- Reagent 1: Cholesterol enzyme reagent
- Reagent 2: Cholesterol standard 200 mg/dl
- Reagent 3: Cholesterol precipitating reagent

Procedure for Cholesterol

Addition sequence	Blank	Standard	Test
Enzyme reagent	1ml	1ml	1ml

Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed well and incubated at 37ºC for 5 minutes. Measure absorbance of the standard and test against reagent blank at 505 nm.

Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 200$$

Result

The amount of cholesterol was found to be _____ mg/dl.

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DEPARTMENT OF BIOCHEMISTRY

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SUBJECT NAME: PRACTICAL-VI -CLINICAL BIOCHEMISTRY
SUB.CODE: 15BCU611 SEMESTER: VI CLASS: III B.Sc (BC)

VIVA QUESTIONS

1. What is the normal level of glucose in blood?
2. Is that glucose eliminated in urine?
3. What is glycosuria?
4. What are the other renal abnormalities in DM?
5. Give the values for post prandial glucose in DM.
6. What is the normal value of blood urea?
7. What are kidney markers?
8. What are liver marker enzymes?
9. What is the normal level of uric acid in blood?
10. What is meant by hypercholesterolemia?
11. What is meant by GOUT?
12. Explain the clinical manifestations of liver markers?
13. What is the normal level of triglycerides in blood?
14. What is the normal level of cholesterol in blood?
15. What is hypoglycemia?
16. What is hyperglycemia?
17. Galactosemia occurs in the deficiency of which enzyme?
18. What are the tests performed qualitatively for the detection of diabetes?
19. Which is the hyperglycemic hormone?
20. Which induces glycogenolysis and inhibit insulin production?
21. Which of the following hormones promote lipolysis?

22. Give the drug of choice for the treatment of primary gout.
23. What is meant by urea clearance?
24. What is the normal specific gravity of urine?
25. What are coagulation enzymes?