KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

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DEPARTMENT OF BIOCHEMISTRY

Subject	**:**	**Practical VI- Clinical Biochemistry and Animal Studies**	**Semester**	**:**	**III**
Subject code	**:**	**17BCP312**	**Class**	**:**	**II M.Sc Biochemistry**

Lecture Plan

S. No	Duration of Period	Name of the Experiments	Support Materials
1	5	Estimation of glucose in serum	T1: 429-430
2	5	Estimation of cholesterol in serum	T1: 417-418
3	5	Estimation of urea in the urine and serum	T2: 124-125
4	5	Estimation of chloride in the urine and serum	T1: 454-455
5	5	Estimation of calcium in the urine and serum	T1: 50-51
6	5	Estimation of magnesium in the urine and serum	T1: 463-464
7	5	Analysis of urinary calculi	W1
8	5	Estimation of Bilirubin in serum (Kit method)	T2: 159-161

9	5	Estimation of triglyceride in serum (Kit method)	T2: 148-150
10	5	Estimation of HDL in serum (Kit method)	T1: 421-423
11	5	Handling of animals	4-6
12	5	Methods of injection	6-8
13	5	Induction of liver toxicity	W2
14	5	Assay of lipid peroxidation in rat liver	W3

Support Materials

T1: Ramnick Sood, 2009. Medical Laboratory Technology (Methods and Interpretations), 6th Edition, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi.

T2: Patel, A.H., 1994. Manual of Medical Laboratory Technology, 1st Edition, Navaneet Prakashan (L) Mumbai, India.

T3: Jann Hau, Steven J. Schapiro, 2010, Handbook of Laboratory Animal Science, 3rd Edition: Essential Principles and Practices, CRC Press.

W1: http://www.merckmanuals.com/professional/genitourinary-disorders/urinary-calculi/urinary-calculi

W2:http://www.hopkinsmedicine.org/healthlibrary/conditions/liver_biliary_and_pancreatic_disorders/alcohol-induced_liver_disease_85,P00656

W3:http://www.researchgate.net/post/Protocol_to_analyze_MDA_TBARS_method_in_homogenated_samples

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(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2017 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : Practical VI: Clinical Biochemistry and Animal Studies
SEMESTER : III
SUBJECT CODE : 17BCP312 CLASS : II M.Sc.(BC)

Course objectives

- To know the analytical methods commonly used in the clinical laboratory.
- To know how can contribute the clinical laboratory to assess the health status of individuals.
- To understand the pathophysiology and molecular basis of the most prevalent diseases.

Course outcome

- Upon successful completion of this course, students will be able to:
- Explain the physiopathological bases and the biochemical markers of the most prevalent diseases in our population.
- Identify the principal analytical procedures used to measure biochemical magnitudes.
- Interpret and integrate the analytical data from the principal biochemical and molecular genetics tests for the screening, diagnosis, prognosis and monitoring of pathologies.

Clinical analysis

1. Estimation of glucose in serum
2. Estimation of cholesterol in serum
3. Estimation of urea in the urine and serum
4. Estimation of uric acid in the urine and serum
5. Estimation of chloride in the urine and serum
6. Estimation of calcium in the urine and serum
7. Estimation of magnesium in the urine and serum
8. Analysis of urinary calculi
9. Estimation of Bilirubin in serum(Kit method)
10. Determination of A/G ratio
11. Estimation of triglyceride in serum (Kit method)
12. Estimation of HDL in serum (Kit method)

ANIMAL STUDIES (Group experiment)

13. Handling of animals
14. Methods of injection
15. Induction of liver toxicity
16. Assay of lipid peroxidation in rat liver.

REFERENCES

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

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

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

Talib, V. H., (2003). A Handbook of Medical Laboratory Technology, CBS Publishers, New Delhi.

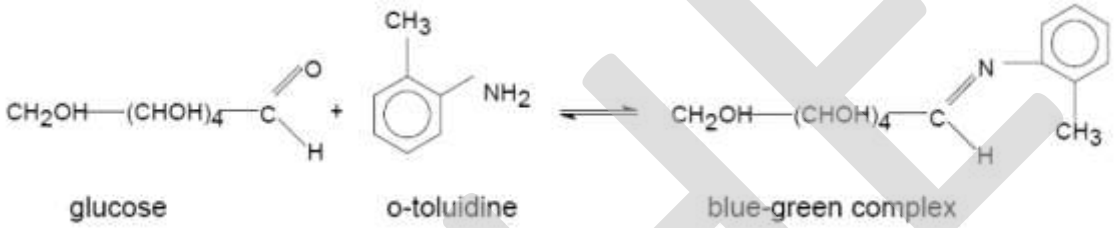
ESTIMATION OF GLUCOSE IN SERUM (O-Toluidine Method)

AIM:

To estimate the amount of glucose present in the given serum and unknown solution.

PRINCIPLE:

The aldehyde group of glucose condenses with o-toluidine in glacial acetic acid (aldehyde free colorless). On heating, giving an embral blue green N- glucosamine which is measured calorimetrically or photo metrically at 630nm. The intensity of color is proportion to the glucose concentration. The presence of thiourea stabilizes the o-toluidine reaction.



REAGENTS:

- **10% TCA:**

  10 gm TCA dissolved in distilled water and made up to 100 ml.

- **O-toluidine reagent:**

  Dissolve 1.5gm pure thio urea in about 200 ml glacial aectic acid analytic grade, using gentle heat if necessary. Transfer to a 1 litre volumetric flask, washing in with glacial aetic acid. Add 60.0ml O-toluidine, mix, make to 1 liter with glacial acetic acid and mix by inversion. Keep in a brown bottle.

- **Stock standard glucose solution:**

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

- **Working Standard (1mg/ml):**

  10 ml of stock standard was diluted to 100 ml of distilled water.

Procedure:

- In a serious of test tubes 0.5, 1.0, 1.5, 2.0, 2.5 ml of standard glucose solution corresponding the concentration 50, 100,150, 200, 250µg respectively.

- **Serum Preparation**: Pipette out 0.2 ml of the given serum sample and unknown solution were mixed with 1.8 ml of 10% TCA, Mix well, allow to stand 10 min at room temperature and centrifuge 10 minutes at 2500 rpm.
- Transfer 1.0 ml of clear supernatant from step 2 in to the new test tube, labelled as test.
- In 2.5 ml of distilled water served as blank.
- To all tubes add 5.0 ml of O-toluidine reagent, Mix by careful lateral shaking
- Heat in boiling water bath for 10 minutes.
- Cool in cold tap water for 4 min.
- Read OD of test and standard at 630 nm.

Normal Value of Blood Glucose

Random : 80-120 mg/dl

Fasting : 60-100 mg/dl

Post prandial : Upto 120mg

CALCULATION

Unknown

The O.D value____ corresponds to ____ µg of glucose.

1.0 ml of unknown solution contains_____ µg of glucose
100ml of unknown solution contains = ___× 100/1000

=_____ mg of glucose.

Serum Sample

The O.D value ____ corresponds to ____ µg of glucose.

1.0 ml of sample solution contains ____ µg of glucose.
0.2 ml of sample solution contains = ___/1.0× 0.2
100ml of sample solution contains = ___ /1000×100

=_____ mg of glucose.

ESTIMATION OF GLUCOSE IN SERUM (O-toluidine Method)						
S.No	**SOLUTION**		**Volume of distilled water (ml)**	**Volume of O-toluidine reagent (ml)**	**Condition**	**Optical density 630nm**
	Volume (ml)	**concentration (µg)**				
Blank	0.00	0.00	2.5			
Standard						
S1	0.5	50	2.0			
S2	1.0	100	1.5			
S3	1.5	150	1.0		Heated in a boiling water bath for 10 minutes	
S4	2.0	200	0.5	5ml		
S5	2.5	250	-			
Unknown						
U1	1.0	_	1.5			
U2	1.0	_	1.5			
Sample						
T1	1.0	-	1.5			
T2	1.0	-	1.5			

Result:

The amount of glucose present in the given serum and unknown solution is_____

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD

AIM:

To estimate the amount of cholesterol in serum and unknown sample.

PRINCIPLE:

Cholesterol in the presence of sulphuric acid it undergoes dehydration to form 3, 5-cholestadiene. This is in turn oxidized and sulphonated to form red coloured cholestapolyene sulphonic acid in the presence of Fe^{3+} ions. The intensity of red color formed is proportional to the amount of cholesterol present in the serum. The colour intensity is measured by using a green filter 540 nm. This reaction has been employed by ZAK'S to estimate the cholesterol in serum and unknown sample.

REAGENTS:

- **Stock Standard Solution:**

  About 100 mg of cholesterol was dissolved and made up to 100 ml with glacial acetic acid.

- **Working Standard:**

  About 10 ml of stock solution was made up to 100 ml with ferric chloride acetic acid reagent (concentration in 100 µg /ml).

- **Ferric chloride of 0.05% in glacial acetic acid**
- **Concentrated sulphuric acid**
- **Glacial acetic acid**

PROCEDURE:

- 0.5 ml to 2.5 ml of working standard were pipetted out into clean test tubes.
- **Serum Preparation:** 0.1 ml of serum was taken and 9.9 ml of ferric chloride reagent was added. Mixed well and kept for 10 minutes at room temperature.
- It was then centrifuged for 10 minutes at 3000 rpm.
- About of 0.5 ml and 1 ml of unknown and serum supernatant was taken in a test tubes. The volume was made up to 5.0 ml with ferric chloride and 3.0 ml of concentrated sulphuric acid were added.

- To prepare the blank, 5 ml of ferric chloride was mixed with 3 ml of concentrated sulphuric acid.
- The test tubes were kept at room temperature for 15 minutes.
- The pinkish red colour formed was measured at 540 nm. Standard graph was drawn for the values obtained.
- From the standard graph the amount of cholesterol present in the unknown and serum sample can be calculated.

Normal Value

Serum cholesterol: 150-200 µg/dl.

CALCULATION

Unknown

The O.D value____ corresponds to ____ µg of cholesterol.

0.5 ml of unknown solution contains_____ µg of cholesterol

100ml of unknown solution contains = ___× 100/0.5×1000

=_____ mg of cholesterol

Serum Sample

The O.D value ____ corresponds to ____ µg of cholesterol.

1.0 ml of sample solution contains ____ µg of cholesterol.

10 ml of serum solution contains___ /0.5 x 10_____µg of cholesterol

0.1 ml of serum solution contains_____ µg of cholesterol

100ml of sample solution contains = ___ /0.1x100/1000

=_____ mg of cholesterol.

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD						
S.No	**SOLUTION**		**Volume of $FeCl_3$(ml)**	**Volume of Conc. H_2SO_4**	**Condition**	**Optical density**
	Volume	**concentration**				

	(ml)	(µg)		(ml)		540nm
Blank	0.00	0.00	5.0			
Standard						
S1	0.5	50	4.5			
S2	1.0	100	4.0			
S3	1.5	150	3.5		Kept at	
S4	2.0	200	3.0		room	
S5	2.5	250	2.5	3ml	temperature	
Unknown					for 15	
U1	0.5	_	4.5		minutes	
U2	0.5	_	4.5			
Sample						
T1	1.0	-	4.0			
T2	1.0	-	4.0			

Result:

The amount of cholesterol present in the given serum and unknown solution is_____

ESTIMATION OF UREA IN THE URINE AND SERUM (DAM- TSC METHOD)

AIM:

To estimate the amount of urea present in blood and in urine.

Principle:

Urea reacts with diacetyl monoxime and thiosemicarbazide in the presence of ferric ions in a hot acidic medium to give a pink coloured compound. Ferric ions are provided by ferric chloride and acidic medium is provided by sulphuric acid and orthophosphoric acid present in acid reagent. The acid reagent is used to condense urea with diacetyl monoxime to yield a yellow coloured complex and further the colour is deepened to pink by the reaction with thioemicarbazide. The formed pink coloured product is spectrophotometrically measured at 540 nm.

REAGENTS

- **Stock Urea Standard Reagent**:

  Dissolve 1g pure dry urea and make up to 100 ml with distilled water. (1 ml= 10 mg)

- **Working Standard**:

  Take 5 ml of stock solution and make up to 100 ml with distilled water (1ml = 500 µg)

- **DAM - TSC Reagent**

  Dissolve 100 mg of diacetyl monoxime, 20 mg of thiosemicarbazide, 900 mg Sodium Chloride in 100 ml of distilled water.

- **Acid Reagent:**

  Add 1ml of orthophosphoric acid and 6 ml of concentrated sulphuric acid to about 75 ml of distilled water, cool the contents and add 0.1 ml of 10% $FeCl_3$ solution. Dilute the solution to 100 ml with distilled water.

- **Serum Preparation:**

  In to a dry test tube 3.4 ml of distilled water and 0.1 ml of blood. Mix well. Pipette 1.5 ml of 10% TCA and mix well. Centrifuged for 10 minutes.

PROCEDURE:

- Pipette out 0.1 ml-0.5ml of the working standard solution (concentration varying from 50-250 µg) into a series of test tubes.
- Make up the given unknown sample to 100 ml with distilled water and pipette out 0.5 ml of this to a test tube.
- To all the test tubes, add distilled water to make up the volume to 0.5 ml. Take 0.5 ml of distilled water in another tube to serve as blank.

- Pipette out 3 ml of DAM-TSC Reagent and 3 ml of acid reagent into all the test tubes and keep at boiling water bath for about 15minutes and cool.
- Note the optical density of the pink colour developed colorimetrically at 540 nm. The intensity of the colour formed is directly proportional to the amount of urea present in it.
- Determine the concentration of the unknown sample from the graph and calculate the urea content in the given sample.

Normal Value

Serum Urea: 10-50 mg/dL

Blood urea Nitrogen 5-25 mg/dL

CALCULATION:

Blood

Colorimetric reading _____ corresponds to _____ µg of urea.

0.5 ml of supernatant contains _____ µg of urea

100 ml of blood contains _____ /1000 x100.

Urine

Colorimetric reading _____ corresponds to _____ µg of urea.

1.5 ml of diluted urine contains _____ µg of urea

100 ml of diluted urine contains _____ /1000 x100

1.0 ml of undiluted urine contain _____/1.0 x 1000 x 100

24hrs of urine sample contains_____ /100 x 1500

ESTIMATION OF UREA DAM-TSC METHOD								
S.No	SOLUTION		Volume of Water	Volume of DAM	Volume of TSC	Volume of Acid	Condition	Optical density
	Volume (ml)	concentration (µg)						

			(ml)	(ml)	(ml)	reagent (ml)		540nm
Blank	0.00	0.00	3.0					
Standar d	0.1	50	2.5					
S1	0.2	100	2.0					
S2	0.3	150	1.5				Keep in	
S3	0.4	200	1.0	1.0	1.0	3.0	boiling	
S4	0.5	250	0.5				water bath	
S5							for 30	
Sample	0.5	_	2.5				minutes	
T1	0.5	_	2.5					
T2								
Urine	1.5		1.5					
U1	1.5		1.5					
U2								

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 _____ g/24 hrs urine.

ESTIMATION OF CHLORIDE IN THE URINE AND SERUM (Van Slyke Method)

AIM:

To estimate the amount of chloride present in urine and serum sample.

PRINCIPLE:

This method uses the Volhard chloride estimation, protein being oxidized by the open Carius method using a solution of silver nitrate in concentrated nitric acid. The organic matter is destroyed and the chloride precipitated at the same the excess silver nitrate in then titrated with standard thiocyanate using ferric alum as the indicator.

REAGENTS:

- **0.05 N Silver nitrate**

  Dissolve 0.895g of silver nitrate in about 2 ml of water and then make up to 100 ml with concentrated nitric acid. The solution keeps in a brown bottle.
- **0.02 N Thiyocyanate**
- **Concentrated nitric acid**
- **5% Ferric alum**

PROCEDURE

Pipette a sample in to clean dry conical flask and run it slowly with constant shaking, 3ml of silver nitrate solution. Add 2 ml of concentrated nitric acid and heat over a Bunsen flame until digestion has occurred and the solution is pale Yellow in colour. If the colour is deep yellow it can be discharged by adding a few drops of saturated potassium permanganate solution and warming again. This should not be necessary with plasma. The digestion usually requires only one to two minutes. When the digestion is completed, cool and add 6 ml of 5% ferric alum. Then titrated with 0.02N thiocyanate until a reddish brown colour persisting for ten to fifteen seconds is obtained. To determine the standard add 2 ml of nitric acid and 6 ml of ferric alum solution to 3 ml of the silver nitrate solution, cool and titrate with 0.02N thiocyanate.

The difference between the two titration gives a measure of the amount of chloride in 1 ml serum and urine in terms of a 0.02 N solution.

CALCULATION:

Serum

Titration value of standard = _____ml

Titre value of serum= _____ml

The amount of chloride in 100 ml of serum = (___ml titration of standard - ____ml titration of the unknown) x 20.

=____mg of chloride

Urine

Titration value of standard = _____ml

Titre value of urine = _____ml

The amount of chloride in 100 ml of urine = (___ml titration of standard - ____ml titration of the unknown) x 20.

=____mg of chloride.

ESTIMATION OF CHLORIDE VAN SLYKE METHOD						
S. No	**SOLUTION**	**Volume of Silver nitrate (ml)**	**Burette Reading**		**Volume of 0.02 N Potassium thiocyanate (ml)**	**Indicator**
			Initial (ml)	**Final (ml)**		
1	Standard	3.0	0.0			
		3.0	0.0			
2	Serum					
	0.5	3.0	0.0			Ferric
	0.5	3.0	0.0			chloride
3	Urine					
	1.0	3.0	0.0			
	1.0	3.0	0.0			

RESULTS:

The amount of chloride present in 100 ml of serum is _____mg of chloride

The amount of chloride present in 100 ml of urine is _____ mg of chloride

ESTIMATION OF CALCIUM IN THE URINE AND SERUM

AIM

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

PRINCIPLE:

The calcium is precipitated as oxalate directly from the serum and after washing, the precipitate is dissolved in acid and titrated with permanganate.

REAGENTS:

- **4% Ammonium oxalate**
- **2% ammonia**
- **0.01 N potassium permanganate**

  Prepare freshly before use by diluting stock 0.1 N solution
- **Sulphuric acid**

PROCEDURE:

To 2 ml of serum add 2 ml of distilled water and 1 ml of 4% ammonium oxalate solution. Mix well and allow to stand overnight at room temperature. Tapered centrifuge tubes are more convenient for this determination. After precipitating the calcium, centrifuge and remove the supernatant fluid without disturbing the precipitate. With tapered tubes the supernatant fluid can be poured off. After doing so stand inverted on a filter paper and allow to drain for five minutes. Centrifuge again, pour off the supernatant fluid, repeat the draining on filter paper, wipe the mouth of the tube and add 2 ml of approximately normal sulphuric acid. Then placed the tubes in water bath for 70-80 ˚C for 5 minutes to dissolve the precipitate removed and titrated the contents hot with 0.01 N $KMnO_4$ solution in a burette till pale pink colour was got which persist for about a minute. Repeat the titration with a duplicates tubes.

Performed a blank titration with 2.0ml normal H_2SO_4. Kept in water bath for 5 minutes and titrated with $KMnO_4$ to a pink colour. The difference between the two tubes tire value gives the volume of 0.01 N $KMnO_4$ required to tire calcium oxalate.

CALCULATION:

Urine

1 ml of 0.01 N $KMnO_4$ is equivalent to 0.2 mg of calcium. Hence since 2 ml of serum is used:

Mg. Calcium per 100 ml serum

= (Titration of unknown – titration of blank) x 0.2 x 100/2

S. No	Volume of Solution (ml)	Burette Reading		Volume of 0.02 N Potassium thiocyanate (ml)	Indicator
		Initial (ml)	Final (ml)		
1	Blank				
	2.0	0.0			
	2.0	0.0			
2	Serum				H_2SO_4
	2.0	0.0			
	2.0	0.0			
3	Urine				
	2.0	0.0			
	2.0	0.0			

RESULT:

The amount of calcium present in the 100 ml of urine is _____ mg of calcium.

ESTIMATION OF MAGNESIUM IN THE URINE AND SERUM (Neill and Neely)

AIM:

To determine the amount of Magnesium in given serum and urine sample.

PRINCIPLE:

Titan Yellow gives a red colour with magnesium Neill and Neely modified earlier methods by using gum ghatti as the colour stabilizer and including calcium in the standard. Calcium intensifies the colour so this allows for the effect of calcium present in the serum.

REAGENTS

- **Sodium tungstate, 10% solution**
- **Sulphuric acid 2/3N**
- **Gum gharrom 0.1 %:** Suspend 0.1 g of powdered gum ghatti in a muslin bag in 100 ml of distilled water for twenty-four hours. The solution keeps well at room temperature.
- **Titan Yellow, 0.05% solution:** Dissolve 0.1 g of the powdered dye in 200ml of distilled water.
- **Sodium hydroxide 4N**
- **Stock standard solution containing 1mg /ml:**

  Dissolve 8.458 grams of $MgCl_2$, $6H_2O$ in distilled water and make up to a litre; or 10.094 grams of $MgNH_4PO_4$, $6H_2O$ in 1N hydrochloric acid and make up to a litre, or dissolve 1.658 grams of reagent grade MgO heated to constant weight at red heat, in 10 to 20 ml. of concentrated hydrochloric acid and make up to a litre with water.
- **Standard solution for use:**

  Dilute 1 ml of stock solution to 200 ml with water. This contains 5 µg. Mg/ ml.
- **Calcium chloride solution, 0.05mg calcium/ml:**

  Dissolve 13.88 mf of Calcium chloride, ($CaCl_2$) in water and make up to 100 ml.

PROCEDURE

Dilute 1 ml of serum with 5 ml of distilled water and precipitate proteins by adding 2 ml of 10% sodium tungstate and 2 ml of 2/3 N sulphuric acid. Centrifuge to 5 ml of the supernatant fluid add in turn 1 ml distilled water, 1 ml of the gum ghatti, 1 ml of 0.05% Titan yellow and 2 ml of 4 N sodium hydroxide. At the same time put up 1 ml of calcium chloride and 5 ml of water and 1 ml of calcium chloride and 2.5 ml of the standard for use plus 2.5 ml of water as blank and standard respectively, completing these in the same way as the test. Read standard and unknown against the blank, using a green filter or with the instrument set at 520 millimicrons.

CALCULATION

Serum

Colorimeteric reading _____ corresponds to _____ µg of magnesium

0.2 ml of serum contains = ____ µg of magnesium

0.2 ml of sample was taken from 5 ml of the supernatant

The reaction mixture will contain

____ x 5/ 1000 x 0.2

1.0 ml serum contains ___ mg of Magnesium

100 ml of serum contains____ x 100

_____ mg of magnesium

Urine

Colorimeteric reading _____ corresponds to _____ µg of magnesium

1.0 ml of sample containing _____ µg of magnesium

100 ml of urine sample contains = ___ x 100 / 1.0 x 1000

=___mg of Magnesium

REAGENTS	**Blank**	**S1**	**S2**	**S3**	**S4**	**S5**	**Serum**		**Urine**	
							S1	**S2**	**U1**	**U2**
Volume of Working standard (ml)	-	0.2	0.4	0.6	0.8	1.0	0.2	0.2	0.2	0.2

Concentration (µg)	-	1	2	3	4	5	-	-	-	-
Volume of water (ml)	2.5	2.3	2.1	1.9	1.7	1.5	2.3	2.3	2.3	2.3
Volume of $CaCl_2$ (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of gum ghatti (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of titan yellow (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
OD at 540 nm										

RESULT

The amount of magnesium present in the given serum sample is_____ mg/ml.

ANALYSIS OF URINARY CALCULI

PREPARATION OF REAGENTS

1. 0.6 N HCl: 5 ml of conc. HCl in 100 ml of water

2. 1.7 M acetic acid: 10 ml of CH_3COOH in 100 ml of water.

3. 0.02 M alcoholic p-Nitrobenzene azo resorcinol: 0.5g in 100 ml ethanol.

4. Magnesium reagent: 0.001% p-Nitrobenzene azo resorcinol in 2N NaOH stored in a polyethylene bolltle in a dark place.

5. Titan yellow: 7.5mg in 100 ml of polyvinyl alcohol

6. 0.3M lead acetate: 10g in 100 ml of water.

PROCEDURE:

Washed the stones to free it from blood, mucous, etc., Stones submitted after sonic disintegration have blood and tissue adhering and or difficult to clean. Placed the stone in a beaker covered with several thickness of gauze held firmly in place with rubber band and washed under running water. Drained and removed the gauze carefully and dried the beaker and stones in an oven. Rinsed tiny crystal with water from squeeze bottle. Recorded the number, weight and dimensions of the stones. Describe briefly the colour and texture of exterior surface. The stones may be photographed for record purpose.

Cut saw or the stone was broken 50 as to examine the interior. Noted whether there was a foreign body which might have acted as a nucleus. A portion of each layer should be crushed and used for separate analysis to identify constituents.

Ashing the portion of the calculus in an open flame can be helpful in the identification of the constituents for flame analysis. To a small portion of the crushed stone was heated in a platinum dish until it glow. If the original bulk was not appreciably changed as ashing and if there was little or no darkening the calculus may be inorganic. If the same charged as was burned almost completely the calculus was found to be organic.

S. No	Experiment	Observation	Interface

1.	Gross appearance	Hard, smooth small or Large small, round white or Gray smooth, small, round Dull stone smooth or rough White gray or yellow white, brown waxy appearance Small, black, light weight stone brittle when dry and Soft and plastic when wet waxy lustrous	Probably calcium oxalate Probably calcium carbonate Probably uric acid Probably phosphate Probably xanthine Probably fibrin Uro stealith Probably cysteine calculi
Chemical analysis of calculi			
S.No	**Chemical group**	**Reagents and treatment**	**Results and interpretation**
1.	Carbonate	To a relatively large sample of pulverised stone in a test tube added approximately 3 ml of 0.6 M HCl	Foaming effervescence carbonate is present
2	Calcium oxalate	Heated HCl solution from the carbonate procedure in order to solubilize the constituents. Cooled and filtered via whatmann No. 1 filter paper (5.5 cm dia) in to another tube added 0.5 ml of saturated sodium acetate and adjusted the pH to appr. 5 with 1.7 Molar CH_3COOH	If a white precipitate forms calcium oxalate is present
Alternate pathway			
1	For oxalate	Heated a small amount of pulverised stone in a porceleain	If effervescence occurs and there is no

		dish. Cooled and placed in a test tubes. Added approximately 2 ml of 0.6N HCl	effervescence in the carbonate procedure oxalate is present. Moderate heat converts to carbonate with upon acidification yield CO_2 prolong intense heat will convert the oxalate in to oxide and invalidate this test
2	Oxalate	To the acid extract added a pinch of MnO_2. The tubes should not be mixed or shaken. It may be necessary to warm the contents of the tubes very slightly to obtain the reaction if only trace quantities of oxalate are present.	Effervescence or tiny bubbles of CO_2 popping upward form the sediment of the tube is seen MnO_2 oxidase the oxalate to CO_2.
3	Non oxalate calcium	If precipitate was formed with CaC_2O_4 procedure filtered using Whatmann No. 1 filter paper. a) If no ppt is noted continued without filtering added 0.5ml of 0.3 Molar potassium oxalate (5.5g/100ml) adjusted the pH to appr. 5 Another method b) Acid extract on microscopic slide with 2 –	If a white precipitate forms no oxalate calcium is present.at pH of 5 oxalate combines with Ca_2 form a insoluble white precipitate of CaC_2O_4 White precipitate or filim reaction is most noticeable if the slide is

		3 drops of saturated $(NH_4)_2C_2O_4$	heated over a dark background
4	Phosphates	Pulverised stone in a test tube and one drop of 10% HCl and 2 drops of H_2SO_4 molibdate reagent and 1 drop of ANSA	Blue colour develops if present in trace amount this occurs upon standing for a few minutes. The blue colour is due to reduced oxides of molybdenum
5	Magnesium and phosphates	If precipitate was formed in the non-oxalate calcium produce filtered through whatmann No.1 filter paper. if no precipitate was noted continued without filtrate added NH_4OH until a pH of 8 was obtained	If the white precipitate forms magnesium and phosphate are present at pH>8. The addition of NH_4 ions to solution contain magnesium and phosphate ions result in the precipitate of NH_4 $MgPO_4$
If no precipitate forms divided the solution into two portions and proceeded as follows			
6	Magnesium	To 1 portion added 0.5 ml of 0.3M Na_2HPO_4 (5.5g / 100ml)	If a white precipitate is formed magnesium is formed
7	Phosphates	To a 2nd portion add 0.5 ml of 0.4 M $MgSO_4$ (5g/100ml)	If a white precipitate is formed phosphate is present
Alternate Procedure for Magnesium and phosphates: Heated a small amount of pulverized stone with 4 ml of 0.6N HCl. Cooled and divided the solution in to two parts. Analyzed 1 portion for magnesium and other for Phosphate.			
	a) Magnesium	Neutralized 1 portion with NH_4OH	

		and added 1 ml of 0.02 M alcoholic para nitro benzene azo resorcinol (0.5g/100ml) ethanol (or) To the acid extract in the test tubes added 2-3 drops of 20% NaOH and 2-3 drops of magnesium reagent	Blue colour forms Magnesium is present Reddish purple reagent slowly becomes a definite blue and (corn flour) Precipitate forms.
	b) Magnesium	Acid extract + 0.2 ml of Titan yellow (7.5mg/100ml) polyvinyl alcohol + 1 ml of 5N NaOH (2g/10ml)	If an orange red colour or precipitate forms magnesium is present
8	Ammonium group	Acid extract was neutralized with 2-3 drops of 2.5 M NaOH + 0.5 ml Nesselr's reagent	Orange brown or rusty precipitate indicates ammonia positive result differentiate NH_4 urates from uric acid
9	Cysteine a) Lead acetate Test	To an small amount of pulverized stone in a evaporating dish added 1-2 drops of 2.5M NaOH and heated. Added several drops of 0.3M lead acetate (10g/100ml) and heated again	Black precipitate is formed probably the present of cysteine is indicated
Sulphides from other sources such as organic matter give a positive test therefore. The presence of a cysteine should be confirmed by the nitroprusside test or by a microscopic examination of crystal.			
	b) Nitroprusside test	Boiled pulverized stone with 2 ml of H_2O. 2 ml of 1M NaCN	Brick red colour was positive reaction which

		(5g/100ml) waited for 5 minutes. Added 3 drops of a freshly prepared solution of sodium nitro prusside (25 mg/ml) H_2O (or) Acid extract + 1 drop of 10% NH_4OH + 1 drop of 5% NaCN waited for 5 minutes. Added 2-3 drops of sodium nitrioprusside solution	may fade to orange red upon standing NaCN converted cysteine to cysteine which then reacts with sodium forms a red colour
10	Urates and uric acids	Pulverized stones in a test tubes + 1 drop of 20% Na_2CO_3 + 2 drops of uric acid reagent (Or) Acid extract was cooled and neutralized with 2.5 M NaOH. Added 1 ml of uric acid reagent	Prompt deep blue colour show uric acid Pale blue colour is negative
Test for differentiating between xanthine and uric acid			
11	Muroxide test uric acid xanthine	Pulverized stone in a test tube + 2 drops of 10% HCl waited for 30 minutes. Added 2 drops of 0.1 % $NaNO_2$ waited for 30-60 minutes added 2 drops of 0.5% NH_4 sulfamate + 2-3 drops sulpha dye reagent	The residue after evaporation is orange with uric acid but lemon yellow with xanthine. After addition of NaOH an orange colour develops becoming red with warming when xanthine is present a cherry red to purple colour develops immediately after addition

			of NaOH when uric acid is present
Bilary calculi			
12	Cholesterol	To the pulverized stone in a test tube added 1 ml of $CHCl_3$ Heated over steam bath allowed to stand until the insoluble material settles, decanted into test tube. To this added 3 drops of acetic anhydride and 1 drop of conc. H_2SO_4 (Extraction can also be done with 3 ml of absolute alcohol)	If the test solution becomes red then blue and finally blue green in colour. It indicates the presence of cholesterol in the calculi
13	Bilirubin	Extracted a portion of the stone with several ml of ethanol added 0.5 ml of diazotised sulfanilic acid	Violet was appeared so bilirubin complexes with diazotized sulfanilic acid to form azobilirubin

ANALYSIS OF URINARY CALCULI IN UNKNOWN SAMPLE

S.No.	Experiment	Observation	Inference
1.	To a relatively large sample of pulverized stone in a test tube added approximately 3.0ml of 0.6N HCl	Effervescence is observed	Presence of carbonate
2.	Heated the HCl solution from the carbonate procedure in order tom solubilize the constituents. Cooled & filteres through whatmann No:	White precipitated is formed	Presence of calcium oxalate.

	1 filter paper (5.5cm india) in tube. Added 0.5 ml of saturated sodium acetate and adjusted the pH to approximately 5 with 1.7M CH_3COOH		
3.	a) Heated a small amount of pulverized stone in a porceleain dish, colled & placed the residue in a test tube added approximately 2.0 ml of HCl. b) To acid extract added a pinch of MnO_2. The tube should not be mixed or shaken. It may be necessary to warm the content of the tube very slightly to obtain the reaction if only trace quantities of oxalate are present.	Effervescence is observed Tiny bubbles of CO_2 popping upward from the sediment of the tube is observed	Presence of oxalate Presence of oxalate is confirmed
4.	Pulverized stone in a test tube + 1 drop of 10% HCl + 2 drops of H_2SO_4 molebdate reagent + 1 drop of ANSA	No blue colour was observed	Absence of Phosphate
5.	Acid extract was neutralized with 2-3 drops of 2.5M NaOH +0.5 ml of nessler's reagent	Orange brown rusty precipitate was formed	Presence of ammonia
6.	To a small amount of pulverized stone in an evaporating dish added 1 or 2 drops of 2.5M NaOH and heated. Added several drops of lead acetate (10mg/10g) and	No black precipitate was formed	Absence of cysteine

	heated again		
7.	Pulverized stone in a test tube + 1 drop of 20% Na_2CO_3 + 2 drop of uric acid reagent	Prompt deep blue colour was observed	Presence of uric acid

RESULT:

The given unknown salt contains carbonate, oxalate, ammonium and uric acid

ESTIMATION OF BILIRUBIN IN SERUM (DMSO/DIAZO Method)

AIM

To estimate the amount of bilirubin present in the given serum or plasma.

PRINCIPLE

Sulfanillic acid reacts with sodium nitrite to form diazotized sulfanillic acid. Total bilirubin reacts with diazotized sulfanillic acid in the presence of DMSO to form azobilirubin, a red purple coloured product in acidic medium.

SAMPLE

Serum (50µl is required)

Even though serum is recommended, plasma from blood treated with heparin can also be used for this test.

REAGENT COMPOSITION

- **Total bilirubin reagent**

  Sulfanilic acid

  HCL

  DMSO
- **Total bilirubin activator**
- **Direct bilirubin reagent**

  Sulfanilic acid

  HCL
- **Direct bilirubin activator**
- **Bilirubin artificial standard**

PROCEDURE

S.No	Reagents	Total bilirubin	
		Sample Blank (µl)	Test (µl)
1.	Total bilirubin reagent	1000	1000
2.	Activator (T/D)	-	20
3.	Serum	50	50
Mix well and incubate for exactly 5 minutes. Measure the absorbance of the sample and standard against the respective sample blank at 546 or 532 nm.			

- Take two test tubes Mark as sample blank and test and add 1000 µl for both tubes.
- Add 20 µl activator for test alone.
- Add 50 µl of serum for all the tubes.
- Mix well and incubate for exactly 5 minutes.
- Measure the absorbance of the sample and standard against the respective sample blank at 546 or 532 nm.

NORMAL RANGE

Total bilirubin : up to 1.2 mg/dl

Direct Bilirubin: up to 0.4 mg/dl

CALCULATION

Total bilirubin = OD of test –OD of sample blank × Factor

$$\text{Total bilirubin concentration} = \frac{\text{OD of test} - \text{OD of sample blank}}{\text{OD of Standard}} \times 10$$

RESULT

The amount of bilirubin present in the given serum sample is_____ mg/ml.

ESTIMATION OF TRIGLYCERIDS IN SERUM (GPO-PAP, End point Assay)

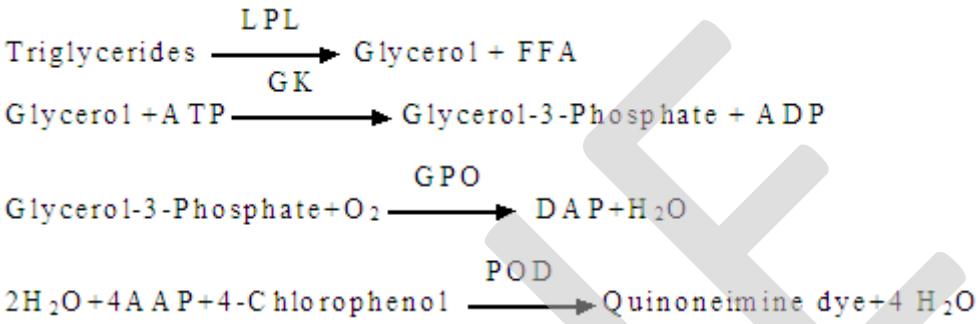
CLASS: II MSC BC COURSE NAME: Practical VI- Clinical Biochemistry & Animal studies
COURSE CODE: 17BCP312 BATCH-2017-2019

AIM

To estimate the amount of triglycerides present in the given serum or plasma.

PRINCIPLE

Triglycerides are hydrolysed by lipoprotein lipase (LPL) to produce glycerol and fatty acids (FFA).In presence of glycerol kinase, ATP phosphorylates glycerol to produce glycerol-3-phosphate and ADP,glycerol-3-phosphate is further oxidized by glycerol-3-phosphate oxidase (GPO) to produce of peroxidase(POD)hydrogen peroxide couples with 4-amino anti pyridine (4 AAP) and 4-chlorophenol to produce red quinoneimine dye, absorbants of coloured dye is measured at 505 nm is proportional to TGL concentration in the sample.



SAMPLE

Serum (10µl is required)

Serum or plasma is collected after 9-12 hours fasting.

REAGENT COMPOSITION

- Triglyceride mono reagent
- Triglyceride Standard

PROCEDURE

Reagents	Blank (µl)	Standard (µl)	Test (µl)
Serum/Plasma	-	-	10
Reagent 2	-	10	-
Reagent 1	1000	1000	1000
Mix well and incubate at 37^{o}C for 10 minutes. Measure the absorbance of the sample and standard			

against the respective sample blank at 505nm.

- Take three test tubes mark as Standard, blank and test
- Add 10 µl of serum/plasma to test, 10 µl of reagent 2 to standard.
- 1000 µl of reagent 1 to all the three tubes.
- Mix well and incubate at 37^{o}C for 10 minutes.
- Measure the absorbance of the sample and standard against the respective sample blank at 505 nm.

NORMAL RANGE

Triglycerides concentration in mg/dl.

Normal: < 150

Border line high: 150-199

High: 200-499

Very high: > 500

CALCULATION

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

RESULT

The amount of triglycerides present in the given serum sample is_____ mg/ml.

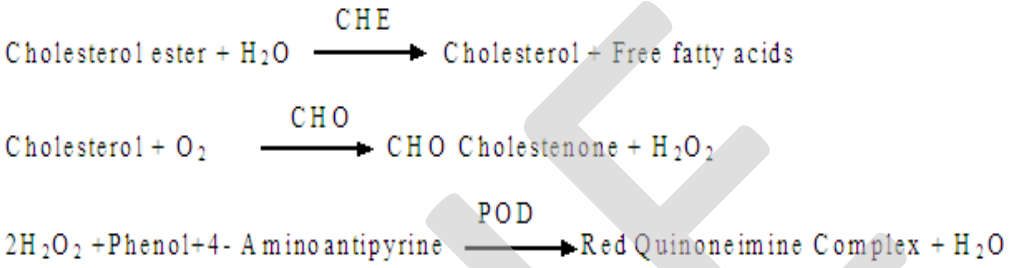
ESTIMATION OF HDL IN SERUM (CHOD/POD METHOD)

AIM

To estimate the amount of HDL present in the given serum or plasma.

PRINCIPLE

The reaction sequence employed in this assay is as follows:



Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolysed by the enzyme Cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidized in the presence of Cholesterol oxidase to liberate Cholest-4 ene-3 one and peroxide. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The intensity of this coloured complex is measured at 505 nm (500-540 nm) and is directly proportional to the cholesterol concentration present in the sample. On addition of the Precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the other Lipoproteins precipitate out.

SAMPLE PREPARATION

Serum (10µl is required)

Take 0.5 ml of serum / plasma in to glass tube. Add 50 µl precipitating reagent. Mix well, leave it at R.T for 10 minutes. Centrifuge at 3000 r.p.m for 10 minutes. Take the clear supernatant for HDL cholesterol estimation.

REAGENT COMPOSITION

- Reagent 1:Cholesterol Enzyme reagent
- Reagent 2: Cholesterol Standard 200 mg/dl.
- Reagent 3:Cholesterol Precipitating reagent (Free)

PROCEDURE

Reagents	Blank	Standard	Test
Enzyme reagent	1 ml	1 ml	1ml
Standard	-	10 µl	-
Supernatant sample	-	-	10 µl
Mix well and incubate at 37°C for 5 minutes. Measure the absorbance of the sample and standard against the respective sample blank at 510 nm.			

- Take three test tubes mark as Standard, blank and test
- Add 1 ml reagent 1
- Add 10 µl of reagent 2 to standard and 10 µl of supernatant.
- Mix well and incubate at 37°C for 90 seconds.
- Measure the absorbance of the sample and standard against the respective sample blank at 510 nm.

NORMAL RANGE

HDL cholesterol

Men : 30-60 mg%

Women: 40-70 mg%

CALCULATION

$$\text{HDL cholesterol mg/dl} = \frac{\text{Absorbance of HDL test}}{\text{Absorbance of Standard}} \times 200$$

RESULT

The amount of HDL cholesterol present in the given serum sample is_____ mg/ml.

ANIMAL STUDIES

HANDLING OF ANIMALS

Animal studies are really recognized fields in which animals are studied in a variety of cross displinary ways. Scholars from fields as diverse as art history, sociology, biology, physiology, literacy studies, geography, philosophy and ferminism, they seek to understand both human animal sections new and in the past and to understand animals as beings inn themselves separate from our knowledge of them.

Some experimental animals:

Mice, rat, Hamster, Guinea pig, Sheep, goat, Chicken, Dog, cat, most perfectly mices and rats are used.

Rats:

Rats should be acclimatized to handling to reduce stress. Rats should handle at the base of the tail using your fingers. Pick up the rats by placing the hand firmly over the back and the rib cage and restraining the head with thumb and fore finger immediately behind the mandibles. Presence of chassaignacs lumbercle, a thin plate of bone extending from the transverse process of the vertebrate makes it difficult to grasp the skin of the back. Holding the rat upside down keeps it distracted and reduces the chances of biting. A variety of resistant devices are available to assist handling the rats.

`Laboratory animals are inevitably subjected to human contact throughout their lives, during both husbandry and experiments. The use of appropriate and skilled handling is essential to ensure that animals readily accept or actively seek human contact and procedures are carried out efficiently. If routine handling procedures are aversive, animals are likely to develop anxiety and show exaggerated stress responses when approached. This is detrimental to animal welfare and will increase the difficulty of handling as animals attempt to avoid contact/restraint and may show defensive aggression. Handling stress can also be a major confounding variable and an unwanted source of variation within and between experiments. Good training in non-aversive handling has benefits for the animal, for the handler and for the reliability of data gained in experiments.

Blood Sampling

Collection of blood from laboratory animals is frequently necessary for a variety of experimental uses including determination of pharmacokinetics, antibody production, clinical pathology evaluation, etc. Blood may be collected from animals which are to survive the procedure or at sacrifice as a terminal event. Whereas there is no limitation on the amount of blood that may be collected terminally, the volume collected from animals surviving the collection is limited to prevent anemia and hypovolemia. As a general guide the 1-3-6 rule should be followed. The rule states that the average blood volume of most laboratory animals is 6% body weight (60 ml/kg); the most blood that can be reasonably expected from a terminal sacrifice is 3% body weight (30 ml/kg); and no more than 1% (10 ml/kg) body weight may be collected during any 2 week period from animals surviving the blood collection. Although venipuncture is generally a satisfactory method for survival blood collection, catheterization of a peripheral vessel may be necessary for animals requiring frequent collection of small quantities of blood.

MICE

There are a variety of methods that are utilized to collect blood from mice. The techniques described below are recommended by OCV staff for survival (tail vein or orbital venous sinus) or terminal blood collection (cardiac).

Lateral tail vein (Saphenous v.) venipuncture

The veins located on the lateral aspect of the mouse's tail are useful for collecting small volumes (< 0.1 ml) of blood. The technique for venipuncture is as described for IV injection except that a small volume of blood is aspirated into the syringe instead of injecting material. The use of a needle without a syringe, allowing the hub to fill with blood, and subsequent collection into a microhematocrit tube is useful when very small quantities of blood are needed.

Orbital venous sinus collection

The sinus surrounding the globe of the mouse's eye is a useful site for collecting larger volumes of blood from surviving animals. The schematic provided in illustrates the location of the sinus. General anesthesia must be provided when collecting from this site. Under general anesthesia the mouse is grasped so that its back rests on the palm of your left hand (right hand if you are left-handed) with its head toward your thumb. The thumb is placed just just lateral to the animal's trachea so that the jugular vein on the same side as the eye from which you are collecting

blood is occluded and the fur on the animals head is drawn into the palm of your hand. This causes the animals eye to proptose (bulge) slightly. A 50 ul microhematocrit tube which has been broken in two is directed into the medial canthus of the eye rotating slightly as the tube is directed to a point directly behind the globe, inserting the non-broken end first. Sufficient pressure must be applied to cut through the fibrous layer which surrounds the sinus. Blood flows through the tube and occasionally around the tube once the sinus has been penetrated. After blood collection, the tube is removed and the eyelids closed and a dry cotton pledget is applied over the eye with gentle pressure to prevent retroorbital hemorrhage. In general blood should not be collected from the same eye more than 3 times, allowing at least 1 week between collections. An antibiotic opthalmic ointment may be applied following bleeding.

Cardiac puncture (diaphragmatic approach)

Cardiac puncture is the preferred technique for terminal collection of large blood volumes. General anesthesia is administered and the animal placed on a solid surface with its ventrum exposed The xyphoid process is palpated at the caudal aspect of the animal's sternum. A notch is present on both sides of this process. Negative pressure should be applied, by placing slight backward pull on the plunger, once it has been inserted beneath the skin. Reflux of blood is apparent once the needle has penetrated the heart.

RATS

Lateral tail vein (Saphaneous v.) vein puncture

The procedure for collecting blood from the rat's tail vein is similar to the technique described for the mouse. A slightly smaller gauge needle (24 ga or larger gauge) can be utilized. Because of the vein's size, larger blood volumes (approximately 1 ml) may be obtained from adult rats.

Orbital venous plexus

The technique describing blood collection from the mouse's orbital venous sinus should be followed for orbital venous plexus collection in the rat. The only difference in that the vessels surrounding the rat's globe are a network of small veins rather than a blood filled sinus and the fibrous connective tissue surrounding the plexus is quite dense. Therefore, the broken end of the hematocrit tube which serves as a cutting edge should be inserted into the plexus. Remember this

technique must be performed under general anesthesia and post bleeding hemostasis is essential to prevent complications.

Cardiac puncture (diaphragmatic approach)

The technique for cardiac puncture from the rat is identical to that described for the mouse except a longer and smaller gauge needle is recommended. A 5 - 10 cc syringe should be used if large blood volumes are desired. This procedure is performed as a terminal event only and general anesthesia is required. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

METHODS OF INJECTION

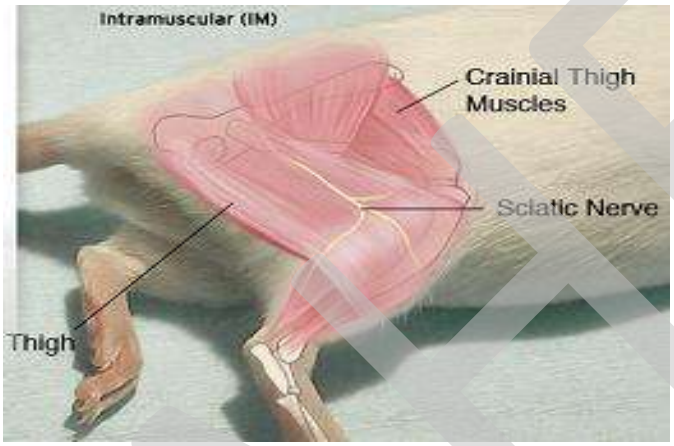
RATS

Subcutaneous

SC injections are performed in rats using the same technique as was described for mice with the following differences. The volume of material administered can be increased to approximately 5 ml per site in an adult rat (>300 grams). Syringe size should be increased proportionately and needles should be 22 ga or larger gauge. May be performed in any area of loose skin along the back or flank. Tenting the skin between the shoulder blades or over the rump creates an appropriate pocket for injection. Inserting the needle under the skin along the flank results in the outline of the needle clearly visible when correctly situated.

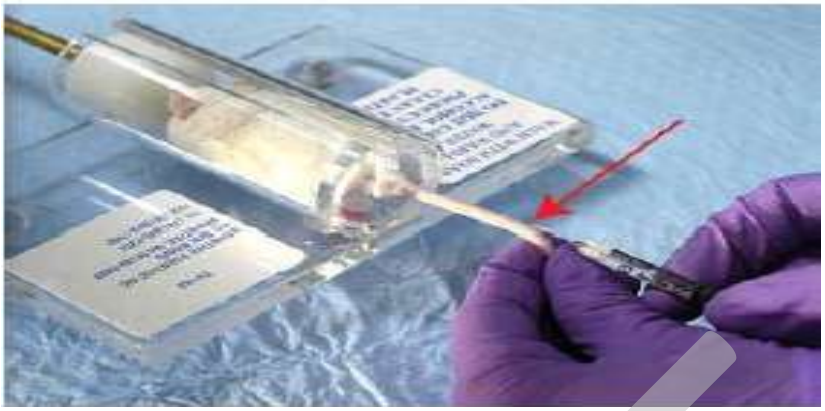
Intramuscular

IM injections may be performed in the rat. Injection volumes are limited to 0.25 ml site because of limited muscle mass. Either the quadriceps muscles located on the cranial aspect of the femur or the hamstrings on the caudal aspect of the femur can be used. Care must be taken to avoid depositing material on or near the sciatic nerve which runs along the caudal aspect of the femur in the thigh. The needle is directed through the skin into the muscle belly approximately 3-4 mm. Aspiration should be attempted before injecting to determine that accidental penetration of a blood vessel has not occured.



Intravenous

IV injection technique for the rat is similar to the mouse. However, the vessels are more difficult to visualize, especially in adult rats. The skin overlying the vessels in adults becomes quite thick, making vascular access much more difficult. For this reason the preferred site for vascular access is near the tail base. Injection volumes administered to an adult rat should not exceed 2 mls and large volumes should be administered slowly to avoid vascular overload. The technique describing IV administration and needle size in mice should be followed. Dilate the blood vessels by warming the mouse. Once warmed, place the mouse in a restrainer. Insert the needle as low as possible towards the tip of the tail, since the vein is very superficial at the tip. The vein will clear from the injection site to the base of the tail if properly situated, whereas ballooning around the injection site will occur if the needle is not properly seated you will note ballooning at the injection site.



Intraperitoneal

The technique for IP injections in rats is virtually identical to mice. Rats should be restrained with their abdomen exposed and their head held downward. The injection site, method and needle size is as described for mice. Because of their larger size < 5.0 mls of material can be administered to an adult rat. Holding the mouse in dorsal recumbency, insert the needle in a position below the bend of the knees; left or right of the midline. Avoid the midline to prevent penetrating the bladder. Angle the needle approximately 45° to the body.

ASSAY OF LIPID PEROXIDATION IN ETHANOL INTRODUCED LIVER TOXICITY IN RATS

Introduction of liver toxicity

Rats are given 40% ethanol (v/v 2.0 ml/100 g body weight) for 30 days.

Principle

In this method, Malondialdehyde and other thiobarbituric acid reactive substance (TBARS) were measured by their reactivity with thiobarbituric acid in the acidic condition to generate a pink colour chromophore which was measured at 535nm.

Reagents

- 0.67 % Thio barbituric acid
- 10% TCA
- Tris HCL buffer

Procedure

The tissue homogenate was prepared in Tris HCL buffer. 1 ml of liver homogenate as combined with 2ml of TBA-TCA-HCL reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling the tubes they were centrifuged at 100 g for 10 minutes and supernatant was taken for measurement. A series of standard solution were treated in a similar mannar. The absorbance of chromophore was measured at 535 nm against a reagent blank that contain no tissue homogenate.

TBARS were expressed as n moles of MDA formed/ mg protein.