M.Sc. NUTRITION & DIETETICS
LAB MANUAL
1st Semester

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MIDNAPORE CITY COLLEGE

Course No. NUD – 195 Experiments on Nutritional Biochemistry

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Unit 9 Experiments on Nutritional Biochemistry I

1. Determination of saponification number:

a. Principle:

Hydrolysis of fat with an alkaline measure in the formation of salt of fatty acid (also called soap) and glycerol. This process is called saponification. From the amount of potassium hydroxide utilize during the hydrolysis, the saponification value of a given fat sample can be calculated. The saponification value is defined as mg of KOH required to saponify 1 g of given fat. The procedure involves refluxing of known amount of fat or oil with a fixed amount of KOH, but on excess of alcoholic KOH, the amount of KOH remaining after hydrolysis is determined by back titrating with standard 0.5(N) Hcl and the amount of KOH utilize for saponification can thus be calculated.

b. Requirements:

i) Fat solvent- A mixture of 95% ethanol and diethyl ether (1:1 v/v)
ii) 0.5 (N) alcoholic KOH- Dissolving 28.5 g of KOH pellets in 20 ml water and making the volume upto 1 lit in a volumetric flask by using 95% ethanol.
iii) 0.5 (N) Hcl
iv) 1% Phenopthalein solution in 95% alcohol.
v) 25 ml burette with stand
vi) 100 ml conical flask
vii) 5 ml pipette
viii) Boiling water bath
ix) Fat sample

c. Procedure:

i. Weight of Blank 50 ml conical flask is measured
ii. Then 1 g of fat sample was taken in that conical flask and weighed
iii. 3 ml of fat solvent and 25ml of 0.5 (N) alcoholic KOH was added to that conical flask and kept in a boiling water bath for 30 minute.
iv. Then cool it at room temperature and add 2-3 drops of phenopthalein into the conical flask.
v. Titrate the solution against 0.5 (N) HCL till the pink color disappears.
vi. Note the volume of HCL from the burette.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**10.1**	**10.1**	**10.1**
2	**10.1**	**11.2**	**10.1**	

e. Result:

Weight of blank conical- 35 g

Conical with fat sample- 36 g

Weight of sample-1 g

$$\text{Saponification value} = \frac{\textbf{28.05 X Titrate value}}{\textbf{Weight of sample}}$$

$$= \frac{28.05X\ 10.1}{1}$$

$$= 283.3\ \text{mg}$$

f. **Inference:**

Here calculated saponification value is 283.30 mg/g of fat. It provides information of the average chain length of the fatty acid in fat. It varies inversely with chain length of fatty acid.

Some fats and their saponification number is given below-

Butter- 210-230 mg/g of fat

Custard oil- 175- 180 mg/g of fat

Safflower oil- 188-198 mg/g of fat

2. Determination of Acid Number

i. Principle:

Different fat sample may contain varying amount of fatty acid. In addition the fat often become rancid during storage and this rancidity is caused by chemical or enzymatic hydrolysis of fats into free fatty acids, which can be determined volumetrically by titrating the sample with KOH. The acidity of fats and oils is expressed as its acid value or number which is defined as mg of KOH required to neutralize the free fatty acid present in 1g of fats and oils. The amount of free fatty acids present or acid value of fat is a useful parameter which gives an indicator about the age and extends of its deterioration.

ii. Requirement:

i. 1% phenopthalein solution in 95% alcohol
ii. 0.01 (N) KOH- 0.56 g of KOH dissolved in distilled water and make the final volume at 1 lit in a volumetric flask
iii. Fat solvent- A mixture of 95% ethanol and diethyl ether (1:1 v/v)
iv. Burette with stand
v. 50ml conical flask
vi. Pipette
vii. Fat sample stored at room temperature

iii. Procedure:

i. A clean and dry 50 ml conical flask was weighed then 1ml fat sample was taken in that conical flask and again weight is measured.
ii. 25ml of fat solvent is added to the conical flask and shake well.
iii. A few drops of phanopthalein is added and the content is mixed well.
iv. The solution is titrated against 0.02(N) KOH until a faint pink colour persists.
v. The volume of KOH in the burette is noted before and after titration.
vi. The same procedure is repeated for two times.

iv. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**8.5**	**14.5**	**6**	**6**
2	**14.5**	**20**	**5.5**	
3	**20**	**26.5**	**6.5**	

v. Result:

Weight of blank conical- 33.01g

Conical with fat sample- 33.88 g

Weight of sample- 0.87 g

vi. Calculation:

$$\textbf{Acid value} = \frac{\textbf{Titrated value X 0.01(N)KOH X Molecular weight of KOH}}{\textbf{Weight of sample (g)}}$$

$$= \frac{6X0.01X\ 56.1}{0.87}$$

= 3.86 mgKOH/g of fat

vii. Interpretation:

Hence the supplied sample contain 3.86mg KOH/g of fat

3. Estimation of Creatinine in blood:

a. Principle:

Creatinin react with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinin. The reaction time and the concentration of picric acid and sodium hydroxide have been optimized to avoid interference from ketoacidosis.

Creatinin + Picric acid ---------- (Alkaline medium) ---------- Orange colour complex

b. Requirement:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample

iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Creatinine kit

c. Procedure:

Reagents/sample	Standard	Test
Working creatinine reagent	500µl	500µl
Standard reagent	50µl	-
Plasma /serum	-	50µl

d. Result:

Absorbance can be measured in colorimeter or by using semiautoanalyser. If the absorbance is measured in colorimeter the total volume of standard and sample should be at least 3 ml. A blank as distilled water or working creatinine reagent will be used in both the cases.

Here the result of colorimetric analysis is given below-

$$\text{Creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X concentration of standard}$$

$$= \frac{0.276}{0.356} X\ 2$$

$$= 1.55\ \text{mg/dl}$$

e. Interpretation:

Reverence value of creatinine level of adult female- 0.6- 1.1 mg/dl
Reverence value of creatinine level of adult male- 0.9- 1.3 mg/dl
The creatinine level of plasma sample is 1.55 mg/dl. So the sample have a high creatinine level.

4. Estimation of uric acid in blood:

a. **Principle:**

Uricase is a very specific enzymeacting on uric acid and the end products are allantoin and peroxide. Peroxidase is used to utilize hydrogen peroxide (proportional to uric acid concentration) to convert chromogen to coloured complex. The intensity of colour produced is proportional to uric acid concentration and is measured photometrically at 500- 550 nm.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{TBHS} \xrightarrow{\text{Peroxidase}} \text{Coloured complex}$$

TBHS- 2,4,6 – Tribromo -3-hydroxybenzoic acid

b. Requirement:

vii. Semiautoanalyser/ Colorimeter
viii. Eppendorf
ix. Plasma or serum sample
x. 1000ml and 100ml micropipette with tips
xi. Distilled water
xii. Uric acid kit

c. Procedure:

i.

Reagents/sample	Blank	Standard	Test
Working reagent	200µl	200µl	200µl
Standard reagent	-	10µl	-
Plasma /serum	-	-	10µl

ii. Mix the above contents well and incubate at 37^0c for 5 minutes

iii. Then add 300 µl of distilled water to the blank, standard and test.

d. Result:

Absorbance can be measured in colorimeter or by using semiautoanalyser. If the absorbance is measured in colorimeter the total volume of standard and sample should be at least 3 ml.

Absorbance of standard- 0.143

Absorbance of test- 0.105

$$\text{Uric acid concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X concentration of standard}$$

$$= \frac{0.105}{0.143} X\ 6$$

$$= 4.40\text{mg/dl}$$

e. Interpretation:

Reference value of uric acid in male is- 3.2- 7 mg/dl
Reference value of uric acid in adult female is- 2.6- 6mg/dl
Uric acid is the end product of purine metabolism and that are catalyzed by uricase enzyme to form allantoin that are excreted through urine. The sample have uric acid value of 4.40mg/dl of uric acid. A serum uric test is done to detect arthritis, gout and kidney disorder patients.

5. Estimation of Serum cholesterol

a. Principle:

Cholesterol ester are hydrolysed by cholesterolesterase (CE) to give free cholesterol and fatty acid. In subsequent reaction, cholesterol oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate cholest 4 en 3 one and hydrogen peroxide. In presence of

peroxidase(POD), hydrogen peroxidase couples with 4 amino antipyrine (4AAP) and phenol to produce red quinineimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample.

Cholesterol ester $\xrightarrow{CE}$ Cholesterol + fatty acids

Cholesterol +O2 $\xrightarrow{CHOD}$ Cholest 4 en 3 one +H2O2

2H2O2 +Phenol +4- AAP $\xrightarrow{POD}$ Quinoneimine dye +H2O

b. Requirements:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Cholesterol kit

c. Procedure:

i.

Reagents/sample	**Blank**	**Standard**	**Test**
Reagent 1	500µl	500µl	500µl
Standard/ reagent 2	-	5µl	-
Plasma /serum	-	-	5µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes

iii. Blank the analyser with reagent blank.

iv. Measure absorbance of standard followed by the test.

v. Calculate results as per given calculated formula.

d. Result:

Absorbance of standard- 0.318
Absorbance of test- 0.142
Standard concentration- 200mg/dl

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X concentration of standard}$$

$$= \frac{0.142}{0.318} X\ 200$$

$$= 89.308 \text{mg/dl}$$

e. Interpretation:

Desirable standard cholesterol level < 200mg/dl
Borderline high risk- 200-239 mg/dl
High risk- >239mg/dl
Serum cholesterol is an important diagnostic criteria that are used to detect the cardiovascular disease risk. The supplied serum sample have 89.30 mg/dl serum cholesterol level which is under the normal range.

6. Estimation of serum triglyceride by kit method:

a. Principle:

Triglyceride are hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acids (FFA). In the presence of glycerol kinase (GK) and Adenosine tri phosphate (ATP), glycerol converts to glycerol 3 phosphate and adenosine di phosphate (ADP). Glycerol 3 phosphate is further oxidized by glycerol 3 phosphate oxidase (GPO) to produce Dihydroxy acetone phosphate (DHAP) and H2O2. In presence of peroxidase (POD), hydrogen produces red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to triglyceride concentration in the sample.

Triglyceride $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Free fatty acids

Glycerol +ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-phosphate+ ADP

Glycerol-3-phosphate +O2 $\xrightarrow{\text{Glycerol-3-phosphate oxidase (GPO)}}$ DHAP +H2O2

2 H2O2 + 4-AAP+4 Chlorophenol $\xrightarrow{\text{Peroxidase (POD)}}$ Quinoneimine dye +4H2O

b. Requirements:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Triglyceride kit

c. Procedure:

i.

Reagents/sample	Blank	Standard	Test
Reagent 1	1000µl	1000µl	1000µl
Standard/ reagent 2	-	10µl	-
Plasma /serum	-	-	10µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes
iii. Blank the analyser with reagent blank.
iv. Measure absorbance of standard followed by the test.
v. Calculate results as per given calculated formula

d. Result:

Absorbance of standard- 0.227
Absorbance of test- 0.104
Standard concentration- 200mg/dl

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X concentration of standard}$$

$$= \frac{0.104}{0.227} X\ 200$$

$$= 91.62 \text{ mg/dl}$$

a. Interpretation:

Desirable standard Triglyceridel level < 150mg/dl
Borderline- 150-199 mg/dl
High risk- 200-499 mg/dl
Very high- >500 mg/dl
Serum triglyceride is an important diagnostic criteria that are used to detect the cardiovascular disease risk. The supplied serum sample have 91.62mg/dl serum cholesterol level which is under the normal range.

7. Estimation of Blood glucose by Glucose oxidase method

a. Principle:

Colorimetric or spectrophotometric estimation of serum glucose using glucose oxidase peroxidase (GODPOD) is a standard method. Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconate. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenol, 4- Aminophenazone (4-AP) in the presence of peroxidase (POD). Peroxidase enzyme acts on hydrogen peroxide to liberate nascent oxygen (O). Nascent oxygen couples with 4 amino antipyrine and phenol to form red quinone dye. The intensity of colour is directly proportional to concentratin of glucose in plasma. The intensity of colour is measured colorimetrically at 530nm.

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{\text{Peroxidase}} \text{Red Quinone dye} + H_2O$$

b. Requirements:

i. GOD POD kit
ii. Test tube
iii. 1000µl and 20µl micropipette with tips
iv. Colorimeter or spectrophotometer
v. Distilled water

c. Procedure:

i. Mixed R1 vowel with 100 ml of R2 and stored at 2-80C in a dark coloured bottle provided with the kit. It will prevent the reagent from light source.

Reagents/sample	Blank	Standard	Test
Reagent 1	1500µl	1500µl	1500µl
Standard/ reagent 2	-	20µl	-
Plasma /serum	-	-	20µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes.

iii.1500µl of distilled water was added to all the test tubes.

iv. Set the colorimeter with reagent blank at 490-500nm.

v. Measure absorbance of standard followed by the test.

d. Result:

Absorbance of standard- 0.13

Absorbance of test- 0.92

Serum glucose concentration (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 100$

$= \frac{0.92}{0.13} X\ 100$

= 707.69 mg/dl

e. Interpretation:

Normal fasting plasma glucose level= 65-110 mg/dl

Post prandial= <120mg/dl

Hyperglycemia is an abnormal condition with excess blood glucose level often associated with diabetes mellitus. The sample have a blood glucose level of 707 mg/dl which is very considered to the normal level. So the patient should follow a strict diet along with follow medical guidelines to reduce the complications of diabetes mellitus.

Unit – 10: Experiments on Nutritional Biochemistry II

1. Estimation of Serum proteins by Biuret method

a. Principle:

Protein contain COO and NH2+ groups joined together by a covalent bond directly to form peptide bond (O=C-N-H). Two or more peptide bond give purple colour when mixed with blue colour alkaline copper solution. Biuret reagent is composed of Sodium potassium turtarate, copper sulphate and NaOH/KOH. When this reagent is mixed with protein solution biuret substance bind with cupric ion and give the purple colour. The number of peptide bond present in protein is dit=rectly proportional to the intensity of the purple colour. Colorimeter or spectrophotometer is used to measure the intensity of the colour in the form of optical density(OD). Thereby OD of unknown serum sample was compared with OD of standard.

b. Requirement:

i. Working biuret reagent
ii. BSA standard (Concentration- 2g/dl= 20mg/ml)
iii. Pipette- 5 ml
iv. 1ml micropipette with tips
v. Test tubes
vi. Serum sample

c. Procedure:

i. Test tubes was marked with S as standard, T as unknown test and B as blank.

Reagents/sample	Blank	Standard	Test
Working biuret reagent	3ml	3ml	3ml
Standard/ reagent 2	-	3ml	-
Plasma /serum	-	-	0.1 ml
Distilled water	3 ml	-	2.9 ml

ii. Mix the above contents well and incubate at 37^0c for 10 minutes.

iii.1500µl of distilled water was added to all the test tubes.

iv. Set the colorimeter with reagent blank at 440-540nm.

v. Measure absorbance of standard followed by the test.

d. Result:

Absorbance of standard- 0.13

Absorbance of test- 0.11

Serum protein concentration (g/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} X\ 2$

$= \frac{0.11}{0.13} X\ 2$

= 1.69 g/dl

e. Interpretation:

The important function of serum is to transport minerals, vitamins and other nutrients from intestine to tissue or organs. Low serum protein causes muscle wasting called marasmus in children. Normal level of serum protein is 6-8 g/dl. So the samples have very low serum protein level of about 1.69 g/dl.

2. Estimation of carbohydrate from food powder

a. Principle:

Starch is hydrolysed by treatment of 6(N) HCL and produce glucose molecule are use for titration after separation of protein by titrating the solution with Barium hydroxide (Ba(OH)2) and Zinc sulphate (ZnSO4).

b. Requirements:

i. Benedicts quantitative reagent (BQR)
ii. 6(N) HCL
iii. 10% Ba(OH)2
iv. 10% ZnSO4
v. 100ml and 50 ml conical flask
vi. 10ml and 5 ml pipette
vii. 100ml volumetric flask
viii. 25 ml burette with burette stand
ix. Bunsen burner
x. Filter paper
xi. Anhydrous Na2CO3
xii. Food powder (Wheat)

xiii. Distilled water

c. Procedure:

a. Hydrolyzation of food starch by acid

i. Supplied food powder was transferred to a 100/250 ml conical flask (1g).

ii. 50 ml of 6(N) HCL was added and the mouth of this flask was plugged by cotton

iii. This conical was placed in a tripod stand under burner for boiling (10-15 min).

iv. After boiling the total set was allow to cool at room temperature and neutralized by Na_2CO_3 unless the frothing is abolished.

b. Titration against BQR

v. Neutralized solution was transferred to 100ml volumetric flask where 10ml of 10% $Ba(OH)_2$ and 10 ml $ZnSO_4$ was added and mixed well.

vi. The volume of the flask was made up to the mark of 100ml and the set was allowed to stand for a few minutes.

vii. The whole solution is filtered and supernatant was collect and transfer to the 25ml burette.

viii. 10ml BQR was transferred to 50ml conical flask & 1 pinch of Na_2CO_3 was added.

ix. The conical was placed over the tripod stand under the burnar.

x. Heat the reagent gently on low flame.

xi. While it started boiling add the sample supernatant from burette drop by drop (drop the solution only during boiling).

xii. Disappearance of blue colour and appearance of pale white precipitate indicate the end point of titration.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**2.8**	**2.8**	**2.8**
2	**2.8**	**5.6**	**2.8**	

e. Result:

10ml BRQ = 20mg glucose

2.8 ml dilute sample contain 20mg glucose

1ml dilute sample contain 20/2.8 mg glucose

100ml dilute sample contain 20×100/2.8 mg glucose = 714.28 mg glucose

1 g food powder contain 714.28 mg glucose.

So, 100g food powder contain 714.28 X 100 mg glucose

So, percentage of glucose is= 71428mg = 71428/1000 = 71.42 g% glucose

f. Interpretation:

According to the Nutritive value of Indian foods by C. Gopalan the carbohydrate content in wheat powder is 71.2 g%. The supplied sample contains 71.42 g% glucose which is similar to the standard value.

3. Estimation of lactose from milk:

a. Principle:

The protein of milk is consists of casinogen, lactoalbumin and lactoglobulin. The protein are precipitated by tungstic acid. Lactose is the only reducing sugar present in milk. Tiltration of milk is essential by the reduction of BQR.

b. Requirements:

i. 10% sodium tungstate
ii. 2/3 (n) H_2SO_4
iii. Anhydrous Na_2CO_3
iv. Benedict quantitative reagent
v. 50 ml and 100ml volumetric flask
vi. 100ml volumetric flask
vii. Filter paper
viii. Burner
ix. Distilled water
x. Supplied milk
xi. Glass beads

c. Procedure:

a. Preparation of protein free titrate

i. 10ml milk was transferred to a 100ml volumetric flask
ii. 10ml of 10% sodium tungstate and 10 ml 2/3 n H_2SO_4 was added to it.
iii. Make the volume upto 100ml by distilled water.

iv. The mixed thoroughly the whole content and allow to stand for 5 minutes.

v. Then filter the solution and taken in a 25 ml burette.

b. Preparation of filtration against BQR

vi. 10ml of BQR was taken in a 50ml conical flask and add about 1 pinch of anhydrous Na2CO3 and 2-3 pieces of glass beads.

vii. The conical flask was then placed over the tripod stand and heat the solution gently over flame and while boiling added the contents from burette drop by drop.

viii. The disappearance of blue colour and appearance of white gelatinous ppt indicates the end point of filtration.

ix. The titration is repeated for three times.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**11**	**15**	**4**	**4**
2	**15**	**18.5**	**3.5**	
3	**18.5**	**23**	**4.5**	

e. Result:

10ml BRQ = 0.0268 g of lactose

4 ml dilute sample reduces 0.0268 g lactose

1ml dilute sample reduces 0.0268/4 g lactose

100ml dilute sample reduces 0.0268×100/4 g lactose = 0.67 g lactose

Here supplied milk is diluted by 10 times.

So the percentage of lactose in supplied milk is 0.67×10 = 6.7g%

f. Interpretation:

According to the standard data available on lactose content in cows milk is about 4.8 %. The supplied sample contains 6.7 g % lactose which is slightly higher than the reference value.

4. Bio chemical testing of food additives:

Chemical testing for the detection of adulterants in food:

Sl No	Name of food	Adulterant	Method of detection
1	Milk	Water	The presence of water can be by putting a drop of milk on a polished slanting surface. The drop of pure milk either or flows lowly leaving a white trail behind it, whereas milk adulterated water will flow immediately without leaving a mark
		Starch	Add a few drops of tincture of Iodine or Iodine solution. Formation of blue colour indicates the presence of starch.
2	Ghee	Vanaspati	Take about one tea spoon full of melted sample of Ghee with equal quantity of concentrated Hydrochloric acid in a stoppered test tube and add to it a pinch of sugar. Shake for one minute and let it for five minutes. Appearance of crimson colour in lower (acid) of Vanaspati or Margarine.
3	Chhana or Paneer	Starch	Boil a small quantity of sample with some water, cool and add a few drops of Iodine solution. Formation of blue colour indicates the presence of starch.
4	Honey	Sugar solution	A cotton wick dipped in pure honey when lighted with a match stick burns and shows the purity of honey. If adulterated, the presence of water will not allow the honey to burn, If it does; it will produce a cracking sound.
5	Turmeric	Metanil yellow	Take ½ teaspoon of the turmeric powder in a test tube. Pour 3 ml of alcohol in the test tube. Mix up the contents thoroughly by shaking the test tube. Add 10 drops of hydrochloric acid it. A pink colouration indicates presence of metanil yellow in the turmeric powder.
6	Mustard seeds	Argemone seeds	Mustard seeds have a smooth surface The argemone seed have grainy and rough surface and are black and hence can be separated out by close examination. When Mustard seed is pressed inside it is yellow while for Argemone seed it is white
7	Chillies powder	Brick powder, salt powder or talc powder.	Take a teaspoon full of chillies powder in a glass of water. Coloured water extract will show the presence of artificial colour. Any grittiness that may be felt on rubbing the sediment at the bottom of glass confirms the presence of brick powder/sand, soapy and smooth touch of the white residue at the bottom indicates the presence of soap stone. To a little powder of chilli add small amount of conc HCl and mix to the consistency of paste,dip the rear end of the match stick into the paste and hold over the flame,brick red flame colour due to the presence of calcium slats in brick powder.

Course No. NUD – 196 Experiments on Physiology & Nutritional Anthropometry

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NUD – 196 Experiments on Physiology & Nutritional Anthropometry

Unit – 11: Experiments on Physiology

1. Determination of clotting time of human blood by capillary tube method

Principle:

Standard initiation is made in the skin of the patient and blood is taken into capillary glass tube. It is the time required for blood to clot normally after bleeding is started. The interval between the moment when bleeding start and the moment when fibrin thread is first seen. Normal value of clotting time is 3-10 minutes. Bleeding time and clotting time is not the same. Bleeding time depends on integrity of platelets and vessel walls whereas clotting time depends on the availability of coagulating factors. In coagulation disorders like hemophilia occurs. Clotting time may be prolonged in the conditions like Vitamin K deficiencies in liver diseases.

Requirements:

i. Fine capillary glass tubes of about 10mm length.
ii. Cotton & spirit
iii. Needle/lancet
iv. Stopwatch

Procedure:

- Make a finger sterile by using spirit
- Puncture the finger by using a lancet to the depth of 3nm
- As soon as blood is visible, start the stop watch.
- Wipe off the first drop of blood and allow the next drop to flow into the capillary tube by introducing one end of the tube into the drop and holding the other end at a lower level
- hold the capillary tube filled blood between the palms so as to maintain it at body temperature.
- After 2 minutes, break off the capillary tube 1-2 cm from one end at every 30 seconds and look for the appearance of thread of fibrin.
- When a thin string of fibrin is seen between the broken ends, stop watch and note the time.
- In the report, maintain the use of capillary method.

Result:

Breaking the 2 cm of blood-filled capillary tube	Time interval	Thread appearance
1st	2 minute	No thread
2nd	After 30 seconds	No thread
3rd	After 30 seconds	Thread appearance

Interpretation:

Normal clotting time by capillary tube method of human blood varies from 2-8 minutes. Here the subjects clotting time is 30 minute. It is between the normal ranges of clotting time.

2. Determination of bleeding time of human blood by duke method using filter/blotting paper:

Principle: A deep skin puncture is made and the length of time required for bleeding to stop is recorded. It determines the function of the platelets and integrity of capillaries.

Equipments:

i. Blotting paper
ii. Stopwatch
iii. Lancet/needle

Procedure:

- Clean the finger tip of the subject with alcohol and allow the skin to dry completely
- Make a deep puncture (blood should flow freely without squeezing) with the help of a sterile lancet.
- Immediately start the stopwatch.
- Blot the drop of blood coming out from the initiation and every 30 seconds by using the blotting paper or circular filter paper. Place the each subsequent drop a little further along the side of the filter paper.
- Stop the stop watch as soon as bleeding ceases.
- Count the number of drops on the filter paper and multiply it by 30 seconds.

Observation and result:

Age- 21 years

Sex- Female

Time	**Blood spot on filter paper**
1st pricking	Spot appearance
After 30 seconds	Spot appearance
After 30 seconds	Spot appearance
After 30 seconds	Spot appearance
After 30 seconds	Spot appearance
After 30 seconds	No spot

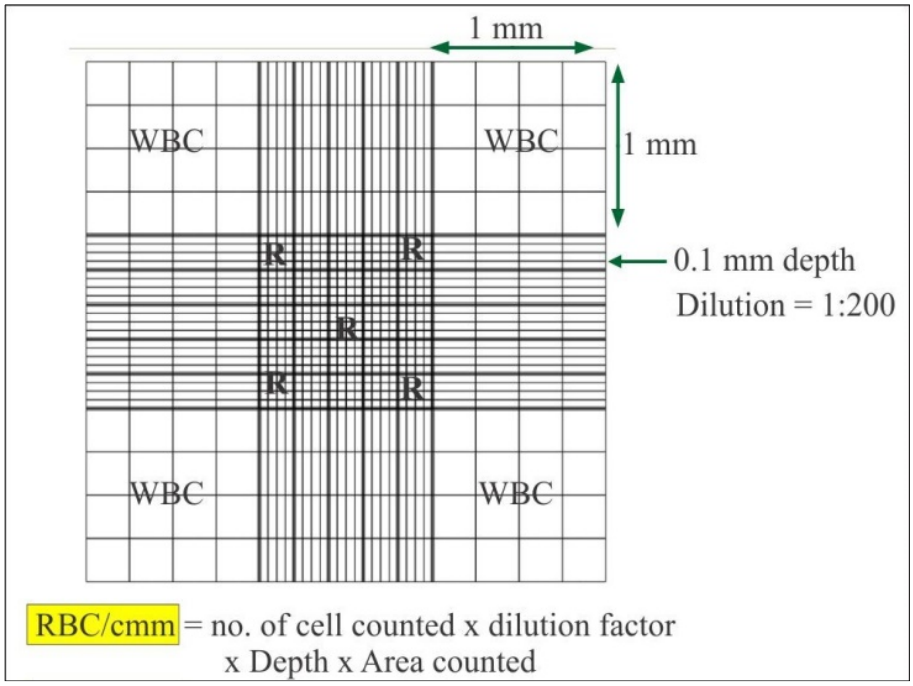
Interpretation:

Normal bleeding time according to Duke method of human blood varies from 1-5 minute. Here, subjects bleeding time is 2.30 minute. It is within the normal range.

3. Enumeration of RBC:

Principle:

The blood specimen is diluted with red blood cells diluting fluid. (usually the concentration is 200 times dilution) which does not remove the white cells but allows the red cells to be counted in known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of red cells /µl of whole blood by using hemocytometer counting chamber under compound microscope. Slide with a rectangular identification creates a chamber. This chamber has a grid of perpendicular lines. It is possible here to count the number of cells in this chamber.



Requirements:

- Microscope
- Hemocytometer
- Sterile needle
- Cotton and alcohol
- RBC diluting fluid

Procedure:

- Clean the hemocytometer and place a cover glass
- At first blood is drawn into the RBC pipette upto 0.5 mark and wipe the extra blood on the surface of the pipette.
- Then immediately immerse the pipette into the RBC diluting fluid and suck the fluid upto 101 mark.

- Dilution should be done very quickly and precisely to prevent clotting of the blood.
- It was collected in an eppendorf tube.
- A few drops of the solution is transferred to the counting chamber below the cover glass.
- Allow the cells to settle down for 2-3 minutes.
- Finally the chamber is placed under microscope and examine the WBC at 100X objective

Observation

Left upper arm- 89
Right Upper arm-85
Left lower arm- 87
Right lower arm- 77
Middle- 82
Total number of RBC - 420

Calculation:

Where (i) Dilution = 1:200 (i.e. 200)

(ii) Area Counted = $\frac{80}{400} = \frac{1}{5}$ Sq. mm.

Since cells are counted in 5 bigger squares and such square is further divided into 16 Small squares. Each small square = $\frac{1}{400}$ sq. mm.

Hence, area of (5 X 16) = 80 such areas = $\frac{80}{400} = \frac{1}{5}$

(iii) Depth of fluid $\frac{1}{10}$ mm.

(iv) Number or red cells counted = N.
Hence, total red blood cells/ cumm

$$M = \frac{N \times 200}{\frac{1}{5} \times \frac{1}{10}} \quad N \times 200 \times 50 = N \times 10{,}000$$

Result:

Dilution- 200

Area= 5 large square X 16 small square = 80 small square

Total small square 16X 25 =400

Area counted= 80/400 =1/5 =0.2

Depth of fluid = 1/10 =0.1

Total RBC (cumm)= $\frac{\text{Number of RBC cells counted X dilution}}{\text{Area counted X depth of fluid}}$

So Total RBC= $\frac{420 \text{ X } 200}{0.2 \text{ X } 0.1}$

= 4200000 /cumm

= $4.2 \text{ X } 10^6$ /cumm

Interpretation:

Normal level of RBC for healthy iman is 4.7 – 6.1 lakh/cumm, Healthy woman is 4.0 – 5.2 lakhs/ cumm. Si, the subjects RBC count is $4.2 \text{ X } 10^6$ /cumm. So the value is under the normal range.

4. Enumeration of WBC:

Principle:

Glacial acetic acid lyses the red blood cells while the gention violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:20 in a WBC diluting fluid and the cells are counted under low power of the microscope by using a counting chamber. The number of cells in undiluted blood are reported per cumm of blood.

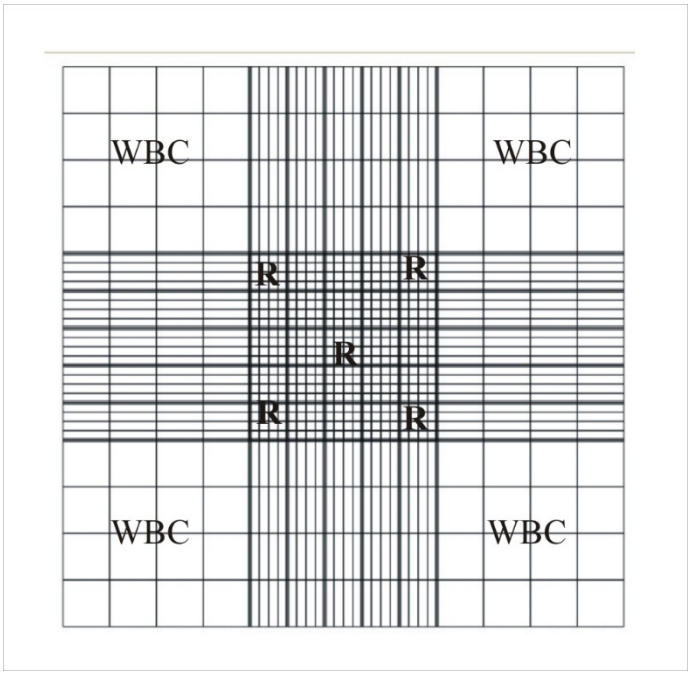
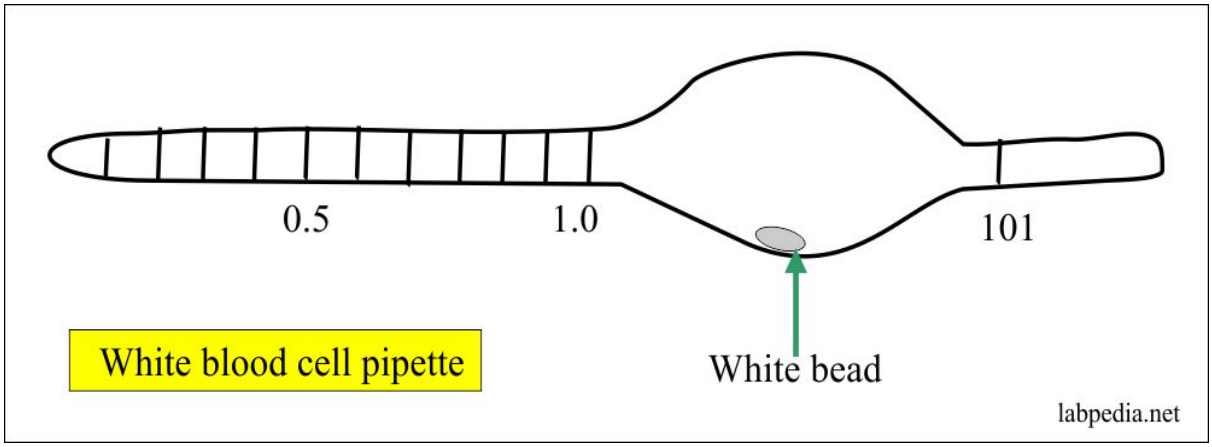


Fig: Neubaur chabmer for count of WBC

Requirements:

- Microscope
- Neubaur chamber
- Sterile needle
- Cotton and alcohol
- WBC diluting fluid



Procedure:

- At first blood is drawn into the WBC pipette upto 0.5 mark and wipe the extra blood on the surface of the pipette.
- Then immediately WBC diluting fluid is drawn upto 101 mark
- After that the solution is rotated for mixing. This result in a dilution of blood to 1:20 ratio.
- Place a cover glass over the chamber and then few drops of the solution is transferred to the counting chamber.
- Allow the cells to settle down for 2-3 minutes.
- Finally, the chamber is placed under microscope and examine the WBC at 40X objective.

Observation:

Left upper arm- 22
Right Upper arm-30
Left lower arm- 39
Right lower arm- 38
Total number of WBC - 129

Result:

$$\text{Number of WBC cells/cumm of blood} = \frac{\text{Number of WBC cells counted X dilution}}{\text{Area counted X depth of fluid}}$$

Where, dilution = 20

Area counted = 4 X 1 sqmm= 4sqmm

Depth of fluid = 0.1 mm

$$\text{Hence, number of cells per cumm} = \frac{129 \text{ X } 20}{4 \text{ X } 0.1}$$

$$= 6450/ \text{ cumm}$$

Interpretation:

The total leucocyte count of the subject is 6450/cumm. The reference value is 4000-11000/cumm. So, the subject has a normal WBC count.

5. Estimation of hemoglobin by cyanmethemoglobin method:

Principle:

Potassium ferricyanide converts the hemoglobin to methemoglobin. The methemoglobin further reacts with potassium cyanide to form a cyanmeth hemoglobin complex. Intensity of the complex formed is directly proportional to the amount of hemoglobin present in the sample.

Requirements:

- Hemocor D solution
- Fresh blood sample
- Colorimeter
- 20 microlitre pipette with tips
- Test tubes
- Hemoglobin standard

Procedure:

- 5ml of hemocor D or drabkins solution is taken in testubes and marked with B as blank, T as test and S a standard.
- Add 20µl of sample of whole blood and 20 µl of hemoglobin standard to the test and standard test tubes respectively.
- Mixed well and incubate at room temperature for 3 minutes.
- Measure the absorbance at 540nm of test and standard solution against B (blank).

Result:

Absorbance of test- 0.23

Absorbance of standard- 0.31

$$\text{Hemoglobin concentration} = \frac{\text{absorbance of test X 251}}{\text{Absorbance of standard X 1000}} \text{ X 60}$$

Where 251 is the dilution factor obtained by, Total reagent volume (5.02ml) /sample volume (0.02ml)

1000 is the multiplication factor to convert mg to g

60 is the concentration of hemoglobin standard.

So, Hemoglobin level = 11.15g/dl

Interpretation:

The normal ranges for hemoglobin depend on the age and, beginning in adolescence, the gender of the person. The normal ranges are:

- Newborns: 17-22 g/dl
- One (1) week of age: 15-20 g/dl
- One (1) month of age: 11-15g/dl
- Children: 11-13 g/dl
- Adult males: 14-18 g/dl
- Adult women: 12-16 g/dl
- Men after middle age: 12.4-14.9 g/dl
- Women after middle age: 11.7-13.8 g/dl

Here the supplied blood is of a adult woman and the content of hemoglobin is 11.15g/dl which is just below the normal level.

6. Determination of blood group and Rh factor

The blood grouping test is based on the heam agglutination reaction. Human RBC contain A and B or AB antigen. Agglutination of RBC with anti A monoclonal and anti B monoclonal or anti AB monoclonal is a positive test indicates the presence of corresponding antigen. Absence of agglutination of RBCs with anti A, anti B or anti AB monoclonal is a positive test indicates the absence of corresponding antigen. Mixing of RBCs with anti D and resulting agglutination indicated the positive Rh factor. Visible agglutination of RBCs with anti D indicates positive reaction where no agglutination with anti D indicates negative reaction and absence of antigen.

Requirements:

- Glass slide
- Disposable sticks
- Anti A, B and D monoclonal kits
- Needle
- Human subject

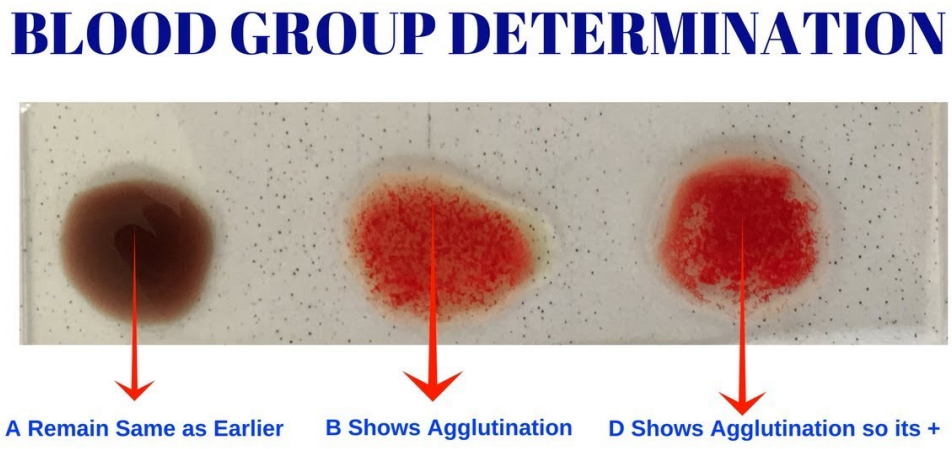
Procedure:

- Take a clean and dry glass slide and placed on a smooth surface.
- Labeled it with anti A, anti B and anti D.
- Drop the Anti A, anti B and anti D monoclonal on the glass slide by maintaining the distance of the antibodies.
- One drop of whole blood will be added to anti A, anti B and anti D monoclonal respectively collected by pricking the needle.
- Mixed up well by a disposable stick and wait for 1 minute.
- Observe the glass slide for evidence of agglutination

Observation:

Reaction with anti A monoclonal	Reaction with anti B monoclonal	Reaction with anti D monoclonal	Blood group
+	-	+	A+
+	-	-	A-
-	+	+	B+
-	+	-	B-
+	+	+	AB+
+	+	-	AB-
-	-	-	O-
-	-	+	O+

Result:



Interpretation: The subjects blood group is B and Rh positive.

7. Determination of pulse rate:

Measurement of arterial pulse (radial) and carotid artery rate of human subjet in different posture like sitting, standing and supine:

Principle:

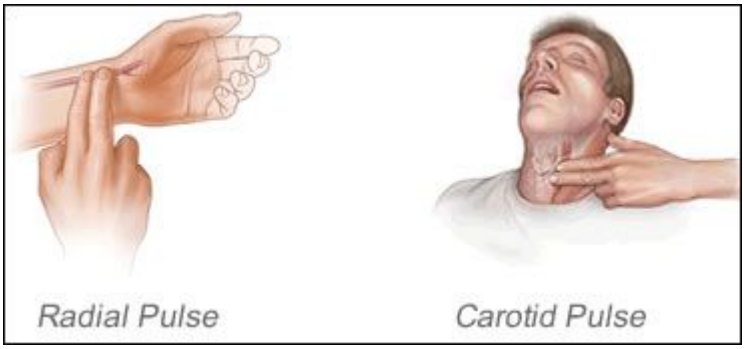
Pulse rate or arterial pulse is defined as the rhythmic expansion of arterial wall due to transmission of pressure waves along the wall of the arteries due to systole of heart, which is about 60-100 beats /minute. For the examination of cardiovascular system arterial pulse is one of the vital sign. Generally arterial pulse was examined from the radial artery or carotid artery.

Requirements:

- Human subject
- Stopwatch

Procedure:

- The subject was asked to take rest for a minimum of 5 minute.
- The radial pulse is detected by gently place the index and middle finger at the base of the thumb and slide down about 2cm in the groove in the wrist, pressing lightly.



- The carotid pulse is detected by gently placing the index and middle finger at the site of esophageal sphincter.
- Count the rate of pulse for one minute.
- This procedure will be applied for measurement of pulse rate on sitting, standing and supine posture.

Observation and Result:

Sitting posture:

No of observation	Radial pulse (Beat/min)	Average (Beat/min)	Carotid pulse (Beat/min)	Average (Beat/min)
1	76	76	74	73
2	76		72	

Standing posture:

No of observation	Radial pulse (Beat/min)	Average (Beat/min)	Carotid pulse (Beat/min)	Average (Beat/min)
1	82	81	87	87
2	80		87	

Supine posture:

No of observation	Radial pulse (Beat/min)	Average (Beat/min)	Carotid pulse (Beat/min)	Average (Beat/min)
1	66	67	66	67
2	68		68	

Interpretation:

On standing posture cardiac output is reduced and stroke volume decreases upto 40%. To compensate cardiac output adrenaline synthesized from adrenal medulla.

On sitting posture normal pulse rate is 70-80 beats/ minute. Here subjects radial pulse rate is 73 and carotid pulse rate is 73 beats/ minute. The pulse rate of the subject is normal.

On standing posture, the subject's radial pulse is 83 beats /minute and carotid pulse is 87 beats /minute which is slightly higher than the normal value.

On supine posture the radial pulse rate and carotid pulse rate is is 67 beats/minute, which is slightly lower than the normal level.

8. Determination of Blood pressure:

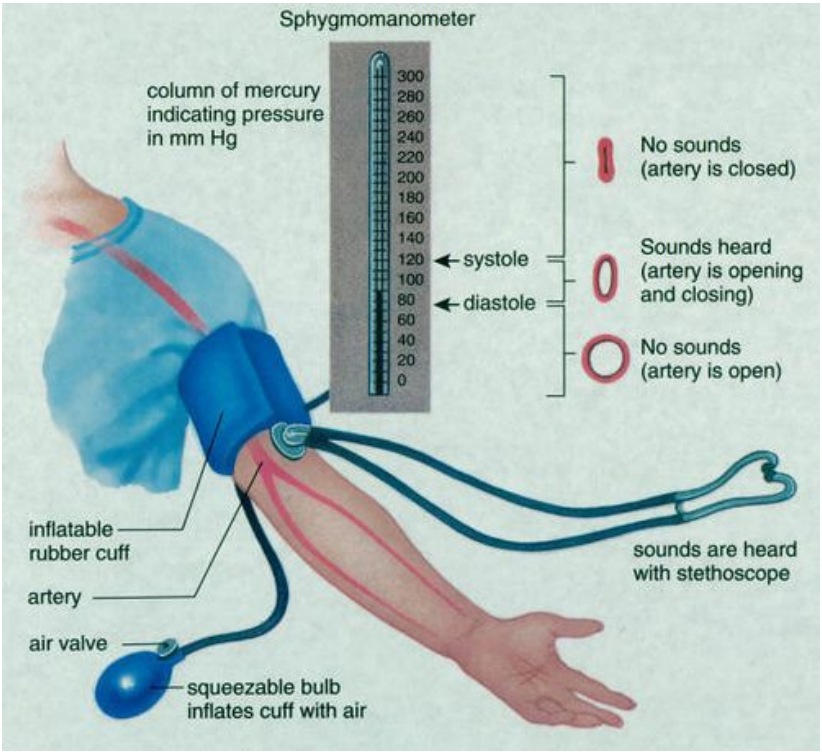
Measurement of blood pressure by sphygmomanometer using mercurial BP monitor or dial type BP apparatus on human subject at different posture like sitting, standing and supine.

Principle:

During flow of blood through the blood vessels a pressure exerted on the wall of the vessels perpendicularly called blood pressure.

The cuff of the sphygmomanometer is wrapped around the arm of the subject. The bag is then inflated until the air pressure in the cuff overcomes the arterial pressure and obliterates the arterial lumen. This is confirmed by palpating the radial pulse that disappears when the cuff pressure is raised above the arterial pressure.

When the pressure in the cuff reaches just below the arterial pressure, blood escapes beyond the occlusion into the peripheral part of the artery and the pulse starts disappearing. This is detected by the appearance of sounds in the stethoscope and is taken as the systolic pressure. Subsequently, the quality of the sound changes and finally disappears. The point where the sound disappears is taken as the diastolic pressure. The sound disappears because the flow in the blood vessels becomes laminar.



Requirements:

- Sphygmomanometer
- Stethoscope
- A sitting chair
- Human subject

Procedure:

- Subject was asked to sit down on the chair and allow to take rest for 5 minutes.
- Exposed the left arm up to shoulder.
- Wrapped the riva-rocci cuff(which consists of inflatable rubber bag attaching to two tubes. 1 tube attached to mercury manometer and other tube is connected to air pump.
- Raised blood pressure of the manometer by compressing the rubber bulb to disappear the radial point.
- Reduce the pressure gradually and noted the level of indications, where pulse reappears. This is systolic pressure.
- Gradually fall the pressure and again pulse disappears and the level of disappearance is noted as the diastolic pressure.
- Blood pressure is measured in this way at standing and supine posture also for 3 times.

Observation and Result:

- Age of the subject- 21 years
- Sex- Female
- Weight- 45 kg

Different posture	No of observation	Systolic BB (mmhg)	Average Systolic BB (mmhg)	Diastolic BP (mmhg)	Average Diastolic BP (mmhg)	Pulse BP (mmhg) (SBP-DBP)	Mean pressure (DBP + 1/3 x PP)
Sitting	1	112	116	66	68	116-68= 48	68+ 1/3 X 48=83
	2	116		68			
	3	120		70			
Standing	1	112	112	62	62	112-62= 50	62+ 1/3 X 50= 78.5
	2	112		62			
	3	112		64			
supine	1	108	110	64	64	110-64= 46	64+ 1/3 X 46= 79
	2	110		62			
	3	112		66			

Interpretation:

Reference normal value of systolic blood pressure is- 110-140mmhg

Reference normal value of diastolic blood pressure is- 60-90 mmhg

Blood pressure changes due to the pressure exert on the wall of the blood vessels mainly depends on the force of contraction of ventricle on ejection of blood. In standing posture the systolic blood pressure falls slightly in respect to the sitting posture. It is due to immediate standing, blood is pooling towards the lower limb due to gravitational force. So venous return is decreased which reduces the systolic blood pressure. After 3-5 minute of standing the blood pressure will return to the normal level by compensatory mechanism of baro receptor. The subjects blood pressure on sitting, standing and supine position belongs to the normal level.

9. Determination of Respiratory Rate:

Principle:

The respiratory system's major functions are to provide an adequate oxygen (O_2) supply to meet the energy production requirements of the body and maintain a suitable acid-base status by removing carbon dioxide (CO_2) from the body. The respiratory rate in humans is measured when a person is at rest and involves counting the number of breaths for one minute by counting how many times the chest rises. Respiratory rate is a vital sign used to monitor the progression of illness and an abnormal respiratory rate is an important marker of serious illness.

Requirements:

- Human subject
- Watch

Procedure:

- At first the subject is asked to take rest for at least five minutes and then lying down
- It is measured by counting the movements of chest and abdominal movements occur during respiration process.
- One complete breath comprises one inhalation, when the chest rises, followed by one exhalation, when the chest falls.
- Count the number of total respiration for a whole minute.

Result:

Age of the subject: 24 years

Sex: Female

Respiratory rate: 17 breath/ minute

Interpretation:

Average resting respiratory rates by age are:

- birth to 6 weeks: 30–40 breaths per minute
- 6 months: 25–40 breaths per minute
- 3 years: 20–30 breaths per minute
- 6 years: 18–25 breaths per minute
- 10 years: 17–23 breaths per minute
- Adults: 12-18 breaths per minute
- Elderly ≥ 65 years old: 12-28 breaths per minute.
- Elderly ≥ 80 years old: 10-30 breaths per minute.

Here the subject's respiratory rate is 17 breaths/ minute which is under the normal value. So the subject may not have may complications related to respiratory system.

10. Determination of Lung function test by using Spirometer

Introduction:

Lung function test or pulmonary function tests (PFTs) are noninvasive tests that show how well the lungs are working. The tests measure lung volume, capacity, rates of flow, and gas exchange. This information can help the healthcare provider diagnose and decide the treatment of certain lung disorders.

A spirometer is used in conjunction with a Windows based computer here for the assessment of PFTs. It is used to determine the dynamic lung function by measuring the Forced Vital Capacity (FVC), Slow Vital Capacity (SVC) and the Maximum Ventilatory Volume (MVV). It has a hand piece which houses a turbine transducer. This hand piece is connected to a computer through a USB interface cable. The software given along with the system is used to record spirometry manoeuvres and to suggest a diagnosis. The computer monitor is used to display the spirometry parameters, the device parameters, information messages and user guide messages. A printer attached to the computer can be used to obtain a hard-copy record of the manoeuvre and the related parameter values.

Indications for use:

1. Diagnosis

• Screening of high risk individuals (e.g. Smokers).

• Measuring the effect of disease on pulmonary function.

• Evaluation of symptoms, signs or abnormal laboratory tests.

• Pre-operative risk assessment.

2. Monitoring

• Assessment of therapeutic interventions (e.g. Bronchodilator/Steroid Therapy for Asthmatics).

• Collateral effects of diseases affecting lung function (e.g. Obstructive Airways Disease, Interstitial Lung Disease, and Congestive Heart Failure).

Parts of the spirometer model Helios 401:

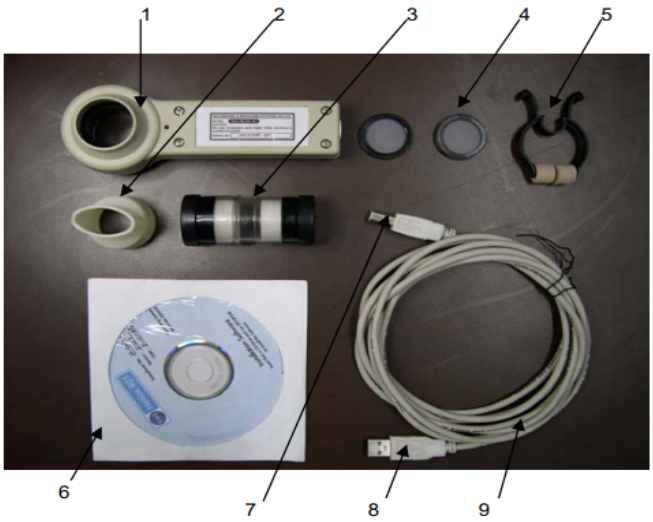


Figure 1: Helios 401 Standard Accessories.

1. Spirometer hand piece
2. Reusable Mouthpiece
3. Transducer, turbine type
4. Air filters
5. Nose clip
6. Software installation CD
7. USB-Serial connector, to be connected to the hand piece
8. USB connector, to be connected to the computer
9. USB-Serial cable

To perform the test, following general procedure should be followed:

- First open software RMS Helios, then click on new patient, enter patient name, age, sex, height, and weight, those which are mandatory must be given*. Click on **Save** and **Exit**.
- Click **Start** the software will ask if the test is being carried pre- or post-medication. The dialog box in Figure 2 will appear. Pre-Medication tests are colour coded as red, Post-Medication tests are colour coded as blue.

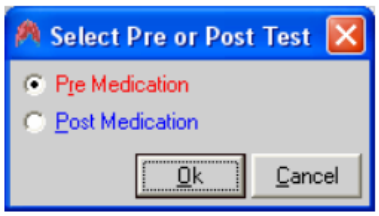


Figure 2: Pre- or Post-Medication Test.

- Select the appropriate category for the test (FVC, SVC or MVV test has been performed) and **click Start**.
- Perform the required test. Once the test has been performed satisfactorily for the software the test will stop and be displayed on screen.
  If you wish to terminate the test before it is completed, click Stop.
- Repeat the test as required until adequate test data has been acquired, or until a maximum of eight acceptable manoeuvres have been performed.
- After the second manoeuvre, and for each succeeding manoeuvre, the software will display a table giving numerical data which allows the user to decide whether the test should be accepted or rejected. Click **Accept** or **Reject**.
- Select the Best Manoeuvre.

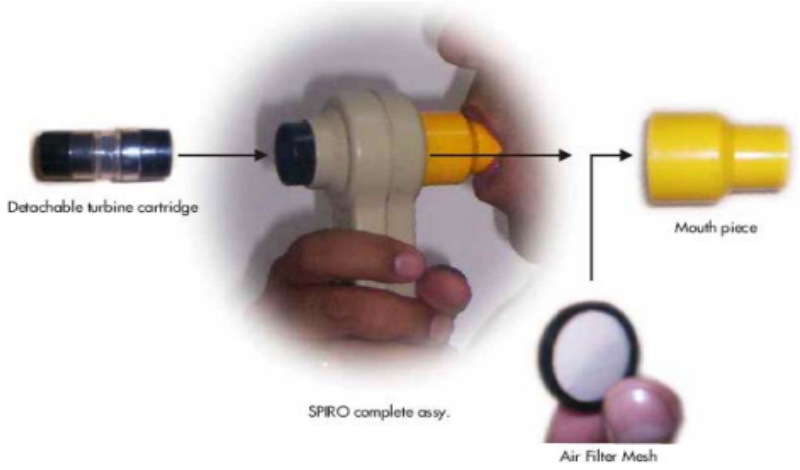


Figure 3: Spirometer in use by a patient

Result:

The numerical values of the various parameters for the particular spirometry test are given in a table to allow comparison between the best pre- and post-medication manoeuvres.

The results from an FVC manoeuvre are shown in Figure 4 below.

Based upon the patient's age, sex, height and weight data, and the spirometry equation selected, numerical values of the parameters are predicted. These are shown in the PRED column. The actual values obtained during a manoeuvre are listed under %PRED as a percentage of the predicted values.

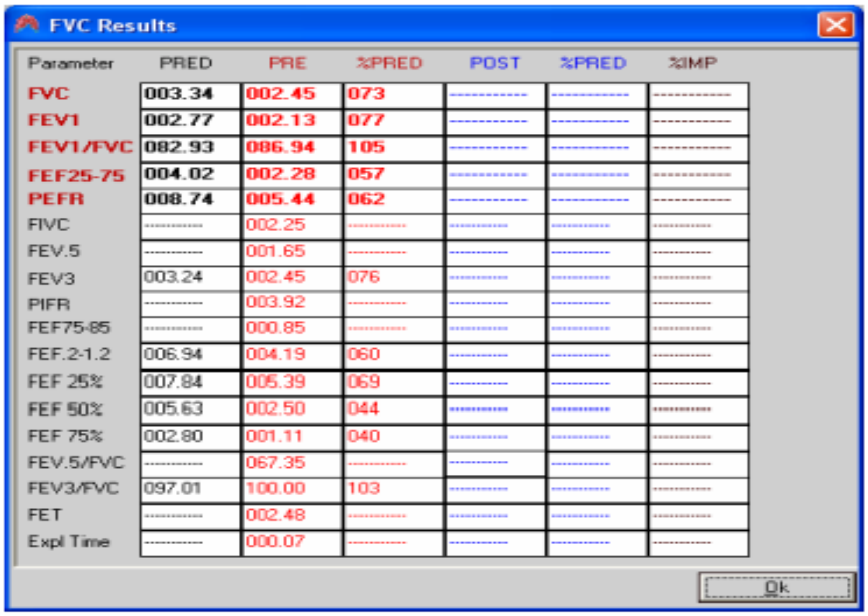


Figure 4: Numerical value display of the results.

Interpretation:

An interpretative result of the FVC manoeuvre is given by plotting the results Depending on where the values of FVC% Pred and (FEV1/FVC)% Pred lie, the patient's lung condition is suggested to be:

- Normal (NORM)
- Restrictive (RES)
- Mixed (MIXED)
- Obstructive (OBS)

Course No. NUD – 196 Experiments on Physiology & Nutritional Anthropometry

Sl no	Contents
Unit – 12: Nutritional Anthropometry	
1.	Introduction of Anthropometry
2.	Nutrition status of Pre-school children using anthropometric parameters
3.	Nutrition status of school going children using anthropometric parameters
4.	Nutritional status of adolescence using anthropometric parameters
5.	Nutrition status of geriatric person.

NUD – 196 Experiments on Physiology & Nutritional Anthropometry

Unit – 12: Nutritional Anthropometry

1. Introduction of Anthropometry

The word anthropometry comes from the Greek word “anthropos” means human and “Methron” means measures refers to the measurement of the human individual of an early tool of physical anthropometry, it has been used for identification, for the purpose of understanding human physical variation.

Anthropometric measurement also have used in epidemiology and medical anthropology foe example in helping to determine the relationship between various body measurement (height, weight, percentage of body fat etc).

Importance of Anthropometric measurement:

The anthropometric measurement is used in nutritional assessment. Those that are used to assess growth and development in infant, children and adolescent include length and head circumference, height, weight, weight for length, MUAC.

Individual measurements are usually compared to reference, standard on a growth chart.

Anthropometric measurements used for adult usually include height, weight, BMI, waist hip ratio and percentage of body fat. This measure is compared to reference standard to assess weight status and the risk of various diseases.

Anthropometric measurement requires precise measuring techniques to be valid.

Length is used for infant those who are unable to stand.

Nutritional growth and metabolic disorder assessment by anthropometric measurement:

Anthropometric data of an individual, group or community, is a major determinant of health like malnutrition, over nutrition, over weight and underweight. Obese and also control obesity which provide useful information to study the relationship among diet nutritional status health. The assessment of nutritional status requires following parameters.

Weight for age: It reflects growth of children. Their weight changes significantly with age, unlike in adults. This index uses as an indicator underweight for child.

Height for age: When a child has under nourished for a long time, the bone growth is affected**.** Low height for the age indicate stunted child.

Weight for height: When a child has been malnourished for a long time, will be short and underweight. A child whose weight for height is low indicates recent weight loss and said wasted child.

Mid Upper Arm Circumference (MUAC): It is used for rapid screening of malnutrition from the 6-59 months.

Body Mass Index (BMI): It is measured by body weight (kg) and body height(m^2) indicate the weight status as underweight, normal weight, overweight, obesity of adult person.

Waist-Hip Ratio: High waist-hip ratio indicate excess abdominal fat or central obesity risk for metabolic disorder.

Anthropometric indicators, site of measurement and Inferences:

Indicator	Site of measurement	Inference
Birth weight	The weight at which the baby is born	It is actually an indicator of maternal nutrition and health status but has implications for the baby's health.
Weight	Measured as weight in kg (to the nearest 100g)	Mainly affected by acute infection and acute food storage. If after the infection the child is an adequate diet weight demonstrates a period of rapid growth (catch-up-growth).
Head Circumference	Measured around the head	Useful in the first 2 years mainly as a measure of brain development
Mid Upper Arm Circumference (MUAC)	It is the **circumference** of the left **upper arm**, **measured** at the **mid**-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium).	MUAC is a measure of adequacy of nutrition. A useful measure for screening acute malnutrition in the community. Also used for patient whose weight/height can't be taken e.g. those who are bed ridden.
Weight for age	Measure of weight compared to the weight of children of the same age and sex from a reference population.	It is indicator of both acute and chronic malnutrition used to identify underweight child.
Height for age	Measure of height compared to the height of children of the same age and sex from a reference population.	It is indicator of chronic malnutrition and is used to identify stunted children.

Weight for height	Weight is below -2SD of expected height of children of same age from a reference population.	It is indicator of malnutrition and is used to identify wasted children.
Stunting	Whose height for age is below minus -2SD (moderate and severe *stunting*) and -3SD (severe *stunting*) from the median of the WHO Child Growth Standards.	It is an indicator of chronic malnutrition
Wasting	Weight is below -2SD of expected Weight of children of same age from a reference population.	It is an indicator of chronic malnutrition
Body Mass Index (BMI)	Weight (kg) divided by height (meter)^2 means $\frac{\text{Weight (kg)}}{\text{Height (m2)}}$	An indicator of nutritional status
Body Surface Area	$\sqrt{\frac{\text{Height (cm)} \times \text{Weight (kg)}}{300}}$	Used mainly for drug prescription for children

2. Nutrition status of Pre-school children using anthropometric parameters.

Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cuboidal fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

Head Circumference: Wrap the tape around the widest possible circumference from the most prominent part of the forehead often (one to two fingers above the eyebrow). Around to the widest part of back of head.

Measured three times and take the longest number.

46cm is the standard head circumference of preschool children according to ICMR 2010.

Chest Circumference: The tape is placed interscapular level posteriorly and anteriorly over the edge of the chest. Measure at the fullest part of your bust, wrap it around (under your armpits, around your shoulder blades, and back to the front) to get the measurement.

32cm is the standard chest circumference of preschool children according to ICMR, 2010.

Result:

Age- 3 years

Sex- Female

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	18.0	18.16 kg
		2	18.5	
		3	18.0	
2	Body height	1	90.0	90.16 cm
		2	90.5	
		3	90.0	
3	Mid Upper Arm Circumference	1	15.0	15.1 cm
		2	15.5	
		3	15.0	
4	Head Circumference	1	49.0	49.33 cm
		2	49.5	
		3	49.5	
5	Chest Circumference	1	48.0	48.16 cm
		2	48.0	
		3	48.5	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal weight of preschool children (3yrs) 12.9 kg.

So, my subjects body weight is 18.16 kg so, my subject's body weight is overweight.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal height of preschool children (3yrs) 99.1 cm.

So, my subjects body height is 90.16 cm so, according to ICMR 2010, my subject's body height is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 15.1 cm. So, according to this chart my subject MUAC is normal.

Interpretation of Head Circumference: The head circumference standard level is 46 cm for preschool children according to ICMR 2010. My subject's head circumference is 49.33 cm which is higher than normal.

Interpretation of Chest Circumference: The chest circumference standard level is 32 cm for preschool children according to ICMR 2010. My subject's chest circumference is 48.16 cm which is higher than normal.

3. Nutrition status of school going children using anthropometric parameters

Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cubital fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

Result:

Age- 6 years

Sex- Male

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	17.0	17.16 kg
		2	17.5	
		3	17.0	
2	Body height	1	110.0	110.33 cm
		2	110.5	
		3	110.0	
3	Mid Upper Arm Circumference	1	14.0	14.16 cm
		2	14.5	
		3	14.0	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal weight of school going children is (6yrs) 25.97 kg.

So, my subjects body weight is 17.16 kg so, my subject's body weight is low.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal height of school going children is (8yrs) 127.22 cm.

So, my subjects body height is 110.33 cm so, according to ICMR 2010, my subject's body height is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 14.16 cm. So, according to this chart my subject MUAC is normal.

4. Nutritional status of adolescence using anthropometric parameters

a. Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

b. Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

c. Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cubital fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

d. Waist Circumference Measurement: At first take the waist circumference measurement of the parameter indicators the status of the abdominal fat deposition. It is helpful for assessment of different type of metabolic disorder. The measurement is taken by the tape horizontally and finally over the midpoint between the terminal end iliac crest particularly the last rib above the iliac crest.

e. Hip Circumference Measurement: It is also good index of body fat distribution. The study associated with waist circumference. The measurement is taken by placing the tape over the area maximum bulge of hip. The end of the tape should meet at the lateral surface of the hip.

f. Waist-Hip Ratio: The waist hip ratio obtained by dividing the waist circumference and hip circumference. So, waist-hip ratio of the subject- $\frac{\text{Waist Circumference}}{\text{Hip Circumference}}$

g. Measurement of skin fold thickness for body fat percentage:

Sites of measurement:

Biceps: Measure midline of the anterior aspect of the arm over the biceps muscle, midpoint on the arm as above.

Triceps: Mark is made in the mid upper arm, mid line of the posterior aspect of the arm over the triceps muscle. Measures with the elbow bend at 90^0 angle for identifying the biceps and triceps. During the measurement the arm should be hanging freely.

Sub scapular:

Found just below and lateral to the bottom tip of the scapula, measured in 45^0 angle. Subjects stand with their arm raised by them. The scapula was hold by the finger tips to find the bottom of the bone. Skinfold thickness is measured in the natural process. Subject should be in relaxed condition**.**

Supra iliac: Found 1 cm above the anterior superior iliac spine (top of the hip bone) in the mid auxillary line (waist line), measured horizontally with the subject breathing normally.

Procedure:

i. Skin fold caliper was used for measuring the skin fold thickness of the four different position of the body I e. biceps, triceps, subscapular and supra iliac.
ii. Measurement was taken from a healthy, undamaged and uninfected dry skin. Moist skin is harder to assess and can influence the measurement.
iii. First mark the skin fold site using a pen.
iv. The skinfold should be firmly grasped by the thumb and index finger using the pads at tip of the thumb and finger. Gently pull the skinfold away from the body.
v. The caliper should be placed perpendicularly to the fold on the site which is marked at approximately 1 cm below the finger and thumb while maintaining the grasp of the skinfold, allow the caliper to be released so that full tension is placed on the skinfold.
vi. Record the measurement in the skin fold caliper.
vii. There are 4 site for the measurement of skin fold for male and female which are biceps, triceps, subscapular and supra iliac.

Result:

Age- 17 years

Sex- Female

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	56.0	56.16 kg
		2	56.5	
		3	56.0	
2	Body height	1	146.5	146.16 cm
		2	146.0	
		3	146.5	
3	Mid Upper Arm Circumference	1	28.0	28.23 cm
		2	28.0	
		3	28.5	
4	Waist Circumference	1	72.5	72.33 cm
		2	72.5	
		3	72.0	
5	Hip Circumference	1	98.0	98.16 cm
		2	98.0	
		3	98.5	
6.	Skin fold thickness (Biceps +Triceps + Subscapullar + Supra iliac)	1	8.6+19.2+18.4+12.7) =58.9	58.9
		2	8.6+19.2+18.4+12.7) =58.9	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal body weight of adolescence is 55.0 kg.

So, my subjects body weight is 56.16 kg so, her body weight is over than normal.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal height of adolescence is 150 cm.

So, my subjects body height is 146.16 cm which is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
5-9 years	<13.5 ≥13.5-14.5 ≥ 14.5	Severe acute malnutrition Moderate acute malnutrition Normal
10-14 years	<16 ≥16.0-18.5 ≥18.5	Severe acute malnutrition Moderate acute malnutrition Normal
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 28.23 cm. So, according to this chart my subject MUAC is normal.

Interpretation of Waist circumference:

Sex	**Cut off value**
Male	85 cm
Female	80cm

Interpretation of Waist-hip Ratio:

This is the cut off value of waist-Hip Ratio

Sex	**Cut off value**
Male	0.89
Female	0.81

My subject's waist hip ratio- $\frac{\text{Waist Circumference}}{\text{Hip Circumference}}$

$= \frac{72.33}{98.16}$ cm

= 0.73cm

So, my subject waist hip ratio is 0.73 cm, which is low.

Interpretation of BMI:

BMI or body mass index provides a reasonable indication of nutritional status of adults. The BMI has good correlation with adiposity. The BMI classification according to WHO-

BMI (Kg/Mt^2)	**Presumptive diagnosis**
<16.0	Chronic energy deficiency (CED) Grade III underweight
16.0-16.99	CED Gread II underweight
17.0-18.49	CED grade I underweight
18.5-24.99	Normal
25.0-29.99	Over weight
30-34.99	Obesity Grade 1
35-39.99	Obesity Grade II
>40	Obesity grade III (Morbid obesity)

My subjects BMI= Weight in Kg/ Height in Mt^2 = 56.16/1.46 = 26.36 kg/mt^2

The subjects BMI is under overweight category according to the classification of BMI by WHO.

Interpretation of Skin fold thickness:

Body Fat	**Male%**	**Female%**
Essential body fat	2-5%	10-15%
Storage	10-15%	10-15%
Total	12-20%	20-30%
Border line	21-25%	31-35%
Obesity	>25%	>33%

THE SUM OF 4 SKINFOLDS (BICEPS, TRICEPS, SUBSCAPULAR, SUPRAILLIAC), BY DURNIN & WOMERSLEY TABLE FOR PERCENTAGE OF BODY FAT:

Skinfold	MEN (age in years)				WOMEN (age in years)			
	17-29	30-39	40-49	50+	17-29	30-39	40-49	50+
15	4.8				10.5			
20	8.1	12.2	12.2	12.6	14.1	17.0	19.8	21.4
25	10.5	14.2	15.0	15.6	16.8	19.4	22.2	24.0
30	12.9	16.2	17.7	18.6	19.5	21.8	24.5	26.6
35	14.7	17.7	19.6	20.8	21.5	23.7	26.4	28.5
40	16.4	19.2	21.4	22.9	23.4	25.5	28.2	30.3
45	17.7	20.4	23.0	24.7	25.0	26.9	29.6	31.9
50	19.0	21.5	24.6	26.5	26.5	28.2	31.0	33.4
55	20.1	22.5	25.9	27.9	27.8	29.4	32.1	34.6
60	21.2	23.5	27.1	29.2	29.1	30.6	33.2	35.7
65	22.2	24.3	28.2	30.4	30.2	31.6	34.1	36.7
70	23.1	25.1	29.3	31.6	31.2	32.5	35.0	37.7
75	24.0	25.9	30.3	32.7	32.2	33.4	35.9	38.7
80	24.8	26.6	31.2	33.8	33.1	34.3	36.7	39.6
85	25.5	27.2	32.1	34.8	34.0	35.1	37.5	40.4
90	26.2	27.8	33.0	35.8	34.8	35.8	38.3	41.2
95	26.9	28.4	33.7	36.6	35.6	36.5	39.0	41.9
100	27.6	29.0	34.4	37.4	36.4	37.2	39.7	42.6
105	28.2	29.6	35.1	38.2	37.1	37.9	40.4	43.3
110	28.8	30.1	35.8	39.0	37.8	38.6	41.0	43.9
115	29.4	30.6	36.4	39.7	38.4	39.1	41.5	44.5
120	30.0	31.1	37.0	40.4	39.0	39.6	42.0	45.1
125	30.5	31.5	37.6	41.1	39.6	40.1	42.5	45.7
130	31.0	31.9	38.2	41.8	40.2	40.6	43.0	46.2
135	31.5	32.3	38.7	42.4	40.8	41.1	43.5	46.7
140	32.0	32.7	39.2	43.0	41.3	41.6	44.0	47.2
145	32.5	33.1	39.7	43.6	41.8	42.1	44.5	47.7
150	32.9	33.5	40.2	44.1	42.3	42.6	45.0	48.2
155	33.3	33.9	40.7	44.6	42.8	43.1	45.4	48.7
160	33.7	34.3	41.2	45.1	43.3	43.6	45.8	49.2
165	34.1	34.6	41.6	45.6	43.7	44.0	46.2	49.6
170	34.5	34.8	42.0	46.1	44.1	44.4	46.6	50.0
175	34.9	-	-	-	-	44.8	47.0	50.4
180	35.3	-	-	-	-	45.2	47.4	50.8
185	35.6	-	-	-	-	45.6	47.8	51.2
190	35.9	-	-	-	-	45.9	48.2	51.6
195	-	-	-	-	-	46.2	48.5	52.0
200	-	-	-	-	-	46.5	48.8	52.4
205	-	-	-	-	-	-	49.1	52.7
210	-	-	-	-	-	-	49.4	53.0

The subjects total skin fold thickness is 58.9cm. As the subject is female and within the age group of 17-29 years old the body fat percentage will be 27.8, which is below the borderline of obesity. So, the person has a normal body fat percentage.

5. Assessment of Nutrition status of geriatric person by anthropometric measurement.

Measurement of Body weight, height, Waist circumference, Waist hip ratio and skin fold thickness was taken to assess the nutritional status.

Result:

Age-70 years

Sex- Male

Body weight- 48.16 (Average for three times)

Body height- 158.16 cm

BMI-30.45kg/mt^2

Waist circumference- 74.33

Hip circumference- 52.16

Waist hip ratio-1.42

Skin fold thickness (total of biceps, Triceps, subscapular and supra iliac) = (6.2+16.1+15.1+11.1) = 48.5cm

Interpretation:

The adult male has BMI of 30.45kg/mt^2 which is grade I obesity. Waist circumference is 74.33 cm which is normal, but Waist hip ratio is 1.42 which is higher than the normal value that indicated obesity, and the person's body fat percentage is 24.7 which is also at the borderline of obesity.