



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Medical Laboratory Sciences Program

Department of Chemical Pathology

Student Practical Log Book

Semester 4

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Student ID:

Batch:

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Instructions

- **Wear gloves when in contact with body fluids, such as serum, plasma, urine or whole blood**
- **Wash your hands when gloves are removed or changed**
- **Perform procedures carefully to minimize aerosol formation**
- **Wear protective clothing such as laboratory coats or aprons when working with specimens**
- **Keep your hands away from your face**
- **Cover all superficial cuts before starting any work**
- **Dispose of specimens and other contaminated materials according to you laboratory's biohazard control procedure**
- **Keep your work area disinfected, disinfect tools and other items that have been in any contaminated area.**
- **Do not eat or drink or apply cosmetics while in the laboratory**

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Practical No (1)

Urine analysis

Quantitative Test for Urine Protein Excretion

By Turbidimetric Method (T.C.A method)

Objectives:

By the end of this practical you should be able to:

- 1- State the principle of TCA.
- 2- Perform the test
- 3- Interpret on your result

Principle:

Trichloroacetic acid(T.C.A) denaturate the urine protein as strong acid and so caused turbidity directly proportional with conc. of protein present in urine sample and can be measured using blue filter(430 nm).

REAGENT:

- 3% T.C.A.

Sample and sampling:

24 hr urine sample collected under certain construction.

Procedure:

REAGENT	BLANK	STD	TEST
3% T.C.A	2 ml	2 ml	2 ml
D.W	0.5 ml	-----	-----
W.STD (100 mg/dl.)	-----	0.5ml	-----
Urine sample	-----	-----	0.5 ml

Mix well; incubate for 5 mins at R.T.

Read the absorption of TEST and STD against BLANK. At 430 nm

Calculation:

$$\frac{OD \text{ of Test}}{OD \text{ of STD}} \times \text{concentration of STD}$$

STD concentration = 100 mg/dl

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Result:

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Reference values:

- 0.05 ---- 0.15 g/dl.
- < 0.4 g/day.

Interpretation:

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Name instructor:

Sign:

Date:

Practical No (2)
Urine analysis
Quantitative Test for Urine Protein Excretion
By Biuret Method

PRINCIPLE:

After removal of protein by using 20% of T.C.A, urine protein react with biuret reagent in alkaline media provided by 1N NaOH, to give a violet colour complex directly proportional with conc. of protein present in urine sample and can be measured using green filter(540 nm).

REAGENT:

- 20% T.C.A.
- 1N NaOH. For removal of acid effect and to provide alkaline media.
- BIURET REAGENT (MAJOR REACTANT).

SAMPLE:

24 hr urine sample collected under certain construction (not diluted).

Procedure:

Step one:-

Into centrifuge tube add 1 ml of 20% T.C.A to 1ml of undiluted urine, mix well, centrifuge for 3 mns.

Decant the supernatant and to precipitate (as sample) into TEST tube:--

REAGENT	BLANK	STD	TEST
1N NaOH	0.5 ml	0.5 ml	0.5 ml
D.W	1.5 ml	0.5	1.5
W.STD (500 mg/dl.)	-----	1ml	-----
BIURET REAGENT	2 ml	2 ml	2 ml

Mix well; incubate for 20 mn at R.T.

Read the absorption of TEST and STD against BLANK. At 540 nm.

CALCULATION:

O.D OF TEST/ O.D OF STD *CONC OF STD = CONC OF TEST (mg/dl.)

Protein excretion (g/day) = conc. of test (mg/dl.)* Urine volume (ml in 24hr)/
100*1000.

Result and interpretation:-

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Normal range:

- 0.05 ----0.15 g/dl.
- < 0.4 g/day.

Comment:-

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Name instructor:

Sign:

Date:

Practical No (3)
Bilirubin estimation
DMSO method

Principle:-

Bilirubin is converted to azobilirubin by diazotized sulfanilic acid and measured photometrically. Of two fractions presents in serum , bilirubin glucuromide and free bilirubin loosely bound to albumin , only the former reacts directly in aqueous solution (direct bilirubin) while free bilirubin requires solubilisation with dimethylsulfoxide (DMSO) to react (indirect bilirubin). In the determination of indirect bilirubin the direct is also determined; the result is corresponding to total bilirubin.

The intensity of the color is proportional to thr bilirubin concentration in the sample.

Reagent

R1 (direct): sulfanilic acid + HCl

R 2 (indirect): sulfanilic acid + HCl + DMSO

R 3: Sodium nitrite

Sample:-

Serum or plasma free of hemolysis and protected from sun light.

Procedure :-

	Blank	Total bilirubin	blank	Direct bilirubin
R 1 (D)	–	–	1.5 ml	1.5 ml
R 2 (T)	1.5 ml	1.5 ml	–	–
R 3	–	0.05 ml	–	0.05 ml
Sample	0.1 ml	0.1 ml	0.1 ml	0.1 ml

Mix and incubate exactly for 5 minutes, read the absorbance at 540 nm.

Result and interpretation:-

(Sample – sample blank) * Factor (to convert to SI unit(micromole/L) multiply by 17.1)

- Factor =.....

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Reference range:-

Total bilirubin up to 1.0 mg/dl

Direct bilirubin up to 0.25 mg/dl

Name instructor:

Sign:

Date:

Practical No (4)

Total plasma protein estimation

Objectives:

By the end of this practical you should be able to:

- 1- State the principle of Total protein colorimetric method.
- 2- Perform the test using the provided procedure
- 3- Interpret on your result and convert into SI unit

Principle:

Copper sulphate of biuret reagent react with peptide bond which linked between the amino acids build up the protein and which cleavage (break down) in strong alkali (NaOH), to form the violet color complex directly proportional to concentration Of protein into sample and read colorimetry at 540 nm (Green filter).

Reagents:

Biuret reagent composed of:-

- 0.2 N NaOH (8 g of sodium hydroxide pills dissolved into 200 ml of D.W)
- 3 g of copper sulphate.
- 9 g of alkaline tartrate (potassium sodium tartrate).
- 5 g potassium iodide.

All completed to 1 liter D.W.

Sample and sampling:

Serum, EDTA plasma.

Procedure:

Tubes	Blank	Test	STD
D.W	1.5 ml	1.4ml	1.4 ml
Sample	–	0.1 ml	-
Standard	–	–	0.1 ml
Biuret reagent	2.5 ml	2.5 ml	2.5 ml

Mix well; incubate at RT for 10 mins.

Read the absorbance of test & STD against blank reagent at 540 nm

Calculation:

$$\frac{OD\ of\ Test}{OD\ of\ STD} \times concentration\ of\ STD$$

STD concentration = 7 g/dl

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Result:

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If the results are to be expressed as S.I units apply $g/dl * 10 = g/l$

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Reference values:

6-8 g/dl

Interpretation:

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Name instructor:

Sign:

Date:

Practical No (5)
Albumin Estimation
Dye binding method (B.C.G)
Colorimetric method: endpoint

Objectives:

By the end of this practical you should be able to:

- 1- State the principle of Dye binding method.
- 2- Perform the test using the provided procedure
- 3- Interpretate on your result and convert into SI unit

Principle:

The method is based on the specific binding of bromocresol green (BCG), an ionic dye dye, and the protein at acid ph with the resulting shift in the absorption wave length of the complex. The intensity of the color formed is proportional to the conc. Of the albumin in the sample;-



Sample and sampling:

Serum, or plasma.

Procedure:

Tubes	Blank	Test	STD
Reagent	1 ml	1 ml	1 ml
Sample	–	0.02 ml	-
Standard	–	–	0.02 ml

Mix well; incubate at RT for 10 mins.

Read the absorbance of test & STD against blank reagent at 600 nm

The color is stable for 30 mn protected from light.

Calculation:

$$\frac{OD \text{ of Test}}{OD \text{ of STD}} \times \text{concentration of STD}$$

STD concentration = 5 g/dl

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Result:

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If the results are to be expressed as S.I units apply $\text{g/dl} * 10 = \text{g/l}$

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Reference values:

3.5-5 g/dl

Interpretation:

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Name instructor:

Sign:

Date:

Practical No (6)

PROTIEN STANDARD CURVE

PROTIEN STANDARD CURVE

You are provided with protein stock standard of 90g/l, construct protein standard curve consist of 4 points (0-9 g/dl). From this curve obtains the concentration of total protein in plasma sample (A).

PROCEDURE:-

1st obtain the points using the formula:-

$Z = A + D(N - 1)$ where:-

Z = FINAL CONC.

A= FIRST CONC.

D= DIFFERENT BETWEEN THE POINT.

N= NUMBER OF POINTS.

2nd prepare the series of dilutions with different conc. Using the formula: RV/O where:-

R= required conc.

V= required volume.

O= original conc.

3rd estimate the plasma total protein level into plasma sample (A) using the prepared series dilutions of standard following the procedure bellow:

reagent	test	Std	blank
Biuret reagent	1 ml	1 ml	1 ml
plasma	0.02	-----	-----
w.std	-----	0.02	-----

Mix well, incubate at R.T for 10 min & read the absorbance of test & STD against reagent blank at 540 nm (green filter).

4th construct on graph paper the standard curve (conc. Versus O.D).

5th obtain the conc. Of the plasma protein into sample (A) from the constructed curve.

Comment on your result.

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Name instructor:

Sign:

Date:

Practical No (7)
Glucose estimation
Enzymatic colorimetric method
End point

Objectives:

By the end of this practical you should be able to:

- 1- State the principle of enzymatic colorimetric method.
- 2- Perform the test using the provided procedure
- 3- Interpretate on your result and convert into SI unit

Principle

In the trinder reaction the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), mixture of phenol and 4-amino antipyrine (4-AA) is oxidized by hydrogen peroxide, to form aredquinoneimine dye proportional to the conc. in the sample.



Sample and sampling;

Serum or heparin plasma free of hemolysis.

Procedure:

	Blank	STD	Test
Color reagent(CR)	1 ml	1 ml	1 ml
Sample	-----	-----	0.02ml
STD	-----	0.02ml	-----

Mix well; incubate for 10 mn at R.T

Read the absorbance of test and STD against reagent blank using 520 nm (green filter).

Calculation:

$$\frac{OD \text{ of Test}}{OD \text{ of STD}} \times \text{concentration of STD}$$

STD concentration = 100 mg/dl

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Result:

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If the results are to be expressed as S.I units in mmol/l (MW. 180)

$$\frac{\text{Mg/dl} \times 10}{\text{M.W}}$$

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Reference values:

F.B.S: 70-----110mg\dl.

R.B.S: 80----120 mg\dl.

2 hour post prandial B.S: 80-----120mg\dl.

Interpretation:

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Name instructor:

Sign:

Date:

Practical No (8)
Glucose tolerance test (oral GTT)

Objectives:

By the end of this practical you should be able to:

- 1- Define GTT.
- 2- Perform GTT using the provided procedure
- 3- Construct GTT curve
- 4- Interpret on your result and convert into SI unit

Definition:

Test done in the biochemical lab to confirm diagnosis of diabetes mellitus or to diagnose other associated disorders (hypoglycemia, malabsorption and thyrotoxicosis - ---etc).

Protocol of doing GTT:

Construct the patient as follows:

- 1- To be fed into carbohydrates diet normally (300 g) for 3 days.
- 2- To be fasting overnight for 10----12 hours. (Allowed to take water).
- 3- Avoid smoking, exercise, shock and stresses.
- 4- At morning when patient coming to the lab. Collect blood and urine samples (fasting samples) into fluoride oxalate container for blood and clean dry container for urine.
- 5- Give the patient standard dose of glucose powder dissolved into water (300mls) according to the age and physiological status as:--
 - For adults; 75 g
 - For children: 1.75g/ kg of body weight.
 - For pregnant: 100 g.
 - You can add flavors (limes...etc) and must be drunken slowly within 5 minutes.
- 6- Collect 1 hour and 2 hours after dose blood and urine samples as mention above.
- 7- Estimate the level of blood sugar using enzymatic kits reagent and detect urine sugar using specific test strips.
- 8- Construct g.t.t curve (concentration versus time).

Interpretation of test results:

- Normal patient response; fasting as normal (70---110mg\dl); 1 hour < 180 mg\dl; 2 hours < or = fasting level. (urine suger is negative)
- Diabetes mellitus response according to severity of disease as fasting > or = 140 mg \dl. (126 mg\dl.) , 1 hour > 200 mg \dl. 2 hours > or =200 mg\dl.(urine sugar is positive in the most of specimens)
- Malabsorption response (flat) as within the same results for fasting, 1 hour and 2 hours after dose respectively.(urine sugar is negative)
- Lag or dumping response (thyrotoxicosis, gastrostomy) as sudden elevation (after 1\2 hour) can reach > 300 mg\dl. And then return to the normal level.(urine sugar is negative)
- Hypoglycemia response as < 70(50)mg\dl.(urine sugar is negative)

Calculation:

$$\frac{OD\ of\ Test}{OD\ of\ STD} \times concentration\ of\ STD$$

STD concentration = 100 mg/dl

Fasting blood glucose concentration =

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1 hr blood

glucose concentration=

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2 hr blood glucose concentration=

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Result:

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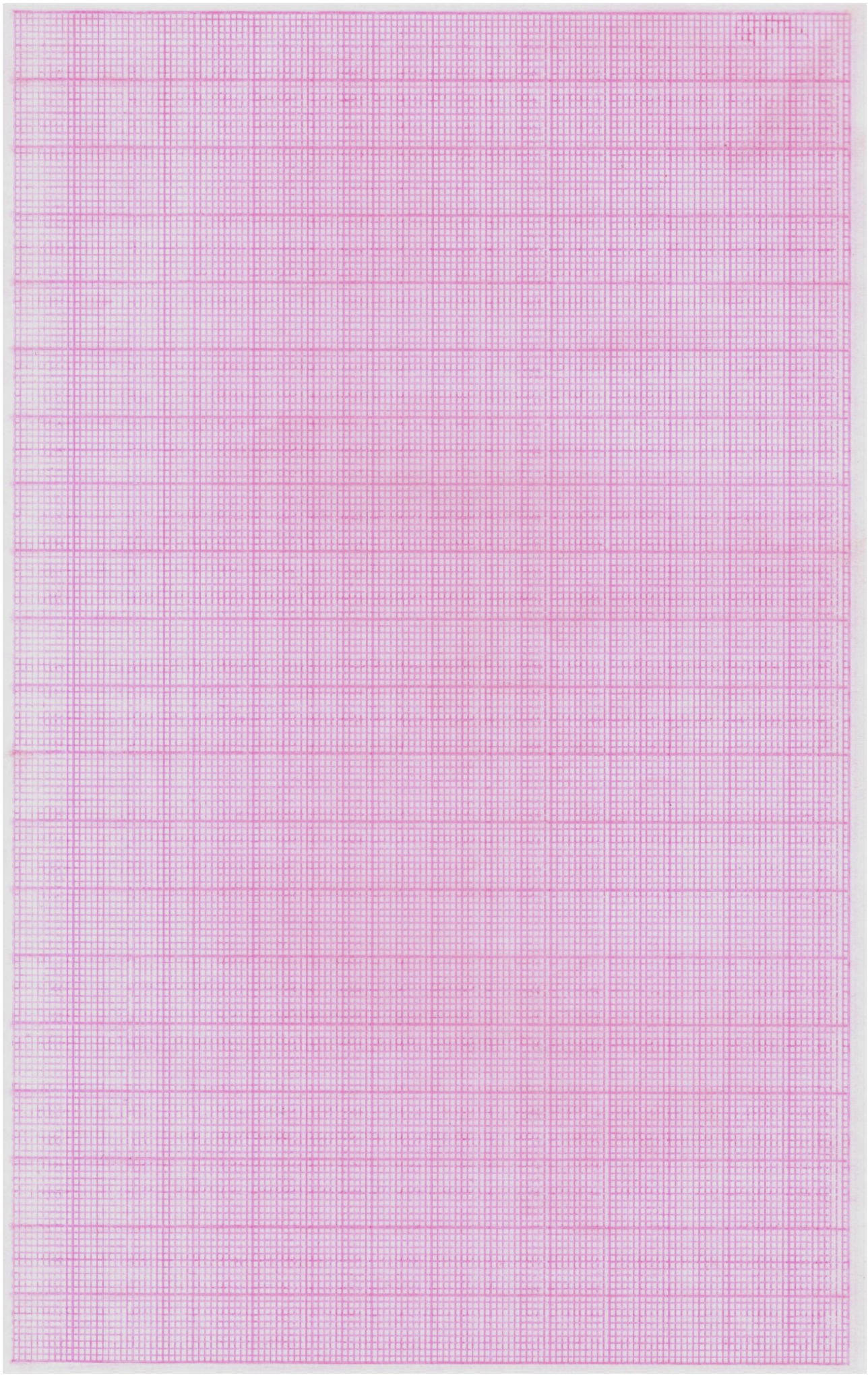
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If the results are to be expressed as S.I units in mmol/l (MW. 180 mg/dl)

$$\frac{\text{Mg/dl} \times 10}{\text{MW}}$$

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.Construct the GTT curve and comment on your curve



Interpretation:

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Name instructor:

Sign:

Date:

Good Luck