National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual-
Clinical Chemistry Department

Second Year, Semester (3)
Clinical Biochemistry-1 (MLS-CCHM-217)

Student Name: ……...

ID: ……**Batch**…….....…...

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

Content

Clinical biochemistry MLS-CCH-216
Practical No (1)
Urinalysis (physical analysis)

Color: ……..

Appearance: ……

Specific gravity: ……..

pH:……

Turbidity: ……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (2)

Urine analysis (chemical tests)

Objectives:

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

1- State the principle of each test.
2- Perform all the tests
3- Interpret on your result

1. Reducing substances:

A- General test by using Benedict's test:

Principle:

Copper sulphate of Benedict reagent reduced by aldehyde group of reducing substance to red cuprous oxide which renders to be change according to the concentration of reducing substances in urine

Requirement:

Instrument:

Water path

Reagent:

Benedict reagent comprised of:

100 g of sodium carbonate

173 g of sodium citrate / in liter of D.W

17.3 g of copper sulphate

Glassware:

Test tube

Sample and sampling:

Fresh urine sample (midstream urine)

Procedure:

1- Boil 2 ml of Benedict reagent to check the efficiency of the reagent

2- Add 4 drops (0.2 ml) of fresh urine

3- Cool down under tap water for 1 min

Result:

1- color doesn't change blue color (Nil) 2-Green color (trace)

3- Green with precipitate (+) 4-Yellow color (++)

5- Orange color (+++) 6-Red color (++++)

Your result:

……..…

Interpretation:

……..……

B- Specific tests:

1 - Glucostix:

Specific for glucose in urine

Principle:

Glucose in urine reacts with the glucose reagent in the glucostrip and the color change according to the amount of glucose.

Requirement:

Glucostrip impregnated glucose reagent (glucose oxidase dry reagent)

Sample and sampling:

Fresh urine sample (midstream urine)

Procedure:

1- insert the strip in the fresh urine

2- take the strip out of urine and wipe out the excess urine

3- Compare the color obtained after 30 – 60 sec with the color given on the strip container.

Your results:

……

Interpretation:

.....

2- Sellwanoff's method:

It is used to detect fructose in urine

Principle:

HCL + fructose ⟶ furfural aldehyde

Furfural aldehyde + resorcinol ⟶ red (pink color)

Requirement:

Instrument:

Water path

Reagent:

1- 33 ml of conc. HCL

2- 50 mg of resorcinol powder

Glassware:

Test tube

Sample and sampling:

Fresh urine sample (midstream urine)

Procedure:

1- Boil 2 ml of sellwanoff's reagent to check the efficiency of the reagent.

2- Add 4 drops (0.2 ml) of fresh urine.

3- Cool down under tap water for 1 min.

Result:

If the fructose present in urine it give pink to red color

Your result:

……

Interpretation:

……

3- Bial's method:

For detection of pentose

Principle:

HCL + pentose ⟶ furfural aldehyde

Furfural aldehyde + orcinol ⟶ red (pink color)

Requirement:

Instrument:

Water path

Reagent:

1- 33 ml of conc. HCL

2- 50 mg of Orcinol powder

Glassware:

Test tube

Sample and sampling:

Fresh urine sample (midstream urine)

Procedure:

1- Boil 2 ml of Bial's reagent to check the efficiency of the reagent.

2- Add 4 drops (0.2 ml) of fresh urine.

3- Cool down under tap water for 1 min.

Result:

If the pentose present in urine it give green color

Your result:

……..

Interpretation:

……..…

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216
Practical NO (3)
Urinalysis (chemical analysis)

Objectives:

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

1- State the principle of each test.
2- Perform all the tests
3- Interpret on your result

1-ketones bodies

Rother's powder: it is used to detect the acetone in the urine.

Principle:

Acetone in the urine reacts with nitroprusside in an alkaline media to give violet color

Requirement:

Reagent:

A mixture of Sodium nitroprusside and sodium carbonate and ammonium sulphate

Other:

Filter paper and paster pipette

Sample and sampling:

Fresh urine sample (midstream urine)

Procedure:

1- Take about 5 mg of powder on the slide or filter paper

2- Add about 5 drops of fresh urine

Result:

If the acetone present in urine, the color convert to violet

Your result:

……..

Interpretation:

……..

2- protienuria:

A- General test:

By using 25% sulfusalisylic acid test

Principle:

Addition of S.S.A to urine contains protein caused turbidity due to reaching of protein isoelectric point of protein & so caused denaturation of protein.

Requirement:

Reagent:

25%S.S.A (25 g of sulfosalisylic acid dissolved in 100 ml of D.W)

Glassware:

Test tube

Sample and sampling:

fresh urine (midstream urine sample)

Procedure:

To 2 ml of fresh urine sample add 4 drops of S.S.A reagent, observe the change

Result:

Turbidity indicates present of protein in urine sample

Your result:

……..

Interpretation:

……..

B-Specific test for albumin:

1-By using conc. nitric acid test

Principle:

Addition of urine contain albumin to conc. nitric acid carefully (by layering) give white ring in the junction between two reagents.

Requirement:

Reagent:

conc. nitric acid

Glassware:

Test tube

Sample and sampling:

fresh urine sample (midstream urine)

Procedure:

To 1 ml of conc. nitric acid in small test tube add 1 ml of urine carefully, observe

Result:

white ring indicates present of albumin

Your result:

……..

Interpretation:

……..

2-albusticks (specific urine test strip):

Principle:

Test strip impregnated by certain dye bind specifically with albumin (BCG or BTB) and give color

Requirement:

Test strip impregnated with BCG

Sample and sampling:

fresh urine sample (midstream urine)

Procedure:

Immerse test strip into fresh urine sample, wipe out the excess urine sample, and match the color obtained within 30-60 sec with colors of the strip container.

Your result:

……..

Interpretation:

……..

Evaluation:

Name and signature of the instructor:

Clinical biochemistry MLS-CCH-216

Practical No (4)

Urinalysis (chemical analysis)

Objectives:

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

1- State the principle of each test.
2- Perform all the tests
3- Interpret on your result

1-Bile pigment:

Mainly bilirubin, bile acid & bile salts

A- fouchet's test:

Principle:

Barium chloride trapped(precipitate sulphate group to which bile pigments is adhered) the bile pigment (yellow color) by filtration or centrifugation and the fouchets reagent, which contain ferric chloride as oxidizing agent, oxidized the yellow bilirubin to green biliverdin which indicates presence of bile in urine.

Requirement:

Reagents:

1-**10% BaCl:** 10 g of BaCl dissolved in 100 ml of D.W.

2-**Fouchet's reagent:** 25 g of T.C.A & 10 g of FeCl dissolved in 100 ml of D.W

Glassware:

Test tube

Sample and samplig:

Fresh urine (midstream urine sample)

Procedure:

To 1 ml of urine add 1 ml of 10% BaCl mix well, centrifuge for 5 mn

Decant supernatant & add few drops of fouchet reagent to the deposit.

Result:

Green color indicates positive result

Your result:

……..

Interpretation:

……..

2\ Tincture iodine:

Principle:

Iodine oxidize the yellow bilirubin into green biliverdin as green ring between the junction of iodine & urine

Requirement:

Reagents:

Iodine crystals dissolved in absolute ethyl alcohol.

Sample and sampling:

Fresh urine sample (midstream urine).

Procedure:

To two mls of urine add carefully (by layering) few drops of tincture iodine without mixing, observe

Result:

Green ring indicates presence of bilirubin.

Your result:

……..

Interpretation:

……..

3-GEMLIN'S TEST:

Principle:

Conc. Nitric acid oxidizes the bilirubin to shade of three layers (green, pink & blue to violet) which indicates positive result.

Reagent:

Conc. Nitric acid.

Sample and sampling:

Fresh urine sample (midstream urine).

Procedure:

To two mls of fresh urine add carefully two mls of conc. nitric acid, observe

Result:

Shade of three layers indicates positive result.

Your result:

……..

Interpretation:

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (5)

Urinalysis

Microscopically examination (urine deposits)

Procedure:

1\ into centrifugal tube take 5 ml of fresh urine sample

2\ centrifuge for 5 mins

3\ decant supernatant

4\ spread the deposit on the glass slide

5\ cover by cover glass

6\ examines under microscope using low power field (X10) then by high power field (X40)

Microscopical examination:

Pus cells: ……

R.B.C.: ……..

Epithelial cells: …….

Mucus Thread: ……..

Casts: ……

Crystals: ……

Parasites: ……..

Yeast: ……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (6)

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 (not diluted).

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:**

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

STD concentration = 100 mg/dl

……..

……..

……..

……..

Result:

……..

……..

Reference values:

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

Interpretation:

……..

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (7)

Total protein

Colorimetric method

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 pilles 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

……..

……..

……..

……..

Result:

……..

……..

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

……..

……..

Reference values:

6-8 g/dl

Interpertation:

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (8)

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;-

BCG+ ALBUMIN $\xrightarrow{\text{PH 4.3}}$ BCG-ALBUMIN COMLEX

Sample and sampling:

Serum, EDTA plasma.

Procedure:

Tubes	Blank	Test	STD
Reagent	3 ml	3 ml	3 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:

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

STD concentration = 5 g/dl

……..

……..

……..

……..

Result:

……..

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

……..

……..

Reference values:

3.5-5 g/dl

Interpretation:

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (9)

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 ared quinoneimine dye proportional to the conc. in the sample.

Beta-D- GLUCOSE + H2O + O_2 **GOD** D- GLUCONATE +H2O2

4-A.A + PHENOL + H2O2 **POD** quinoneimine +H2O

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:

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

STD concentration = 100 mg/dl

……..

……..

……..

……..

Result:

……..

……..

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

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

……

……..

……..

……..

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:

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-216

Practical No (10)

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 alter associated disorders (hypoglycemia, malabsorption and thyrotoxicosis ----etc).

Protocol of doing GTT:

Construct the patient as follows:

1- To be feed 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 suger 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 =

……..

……..

……..

1 hr blood glucose concentration=

……..

……..

……..

2 hr blood glucose concentration=

……

……

……

Result:

……..

……..

……..

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

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

……

……..

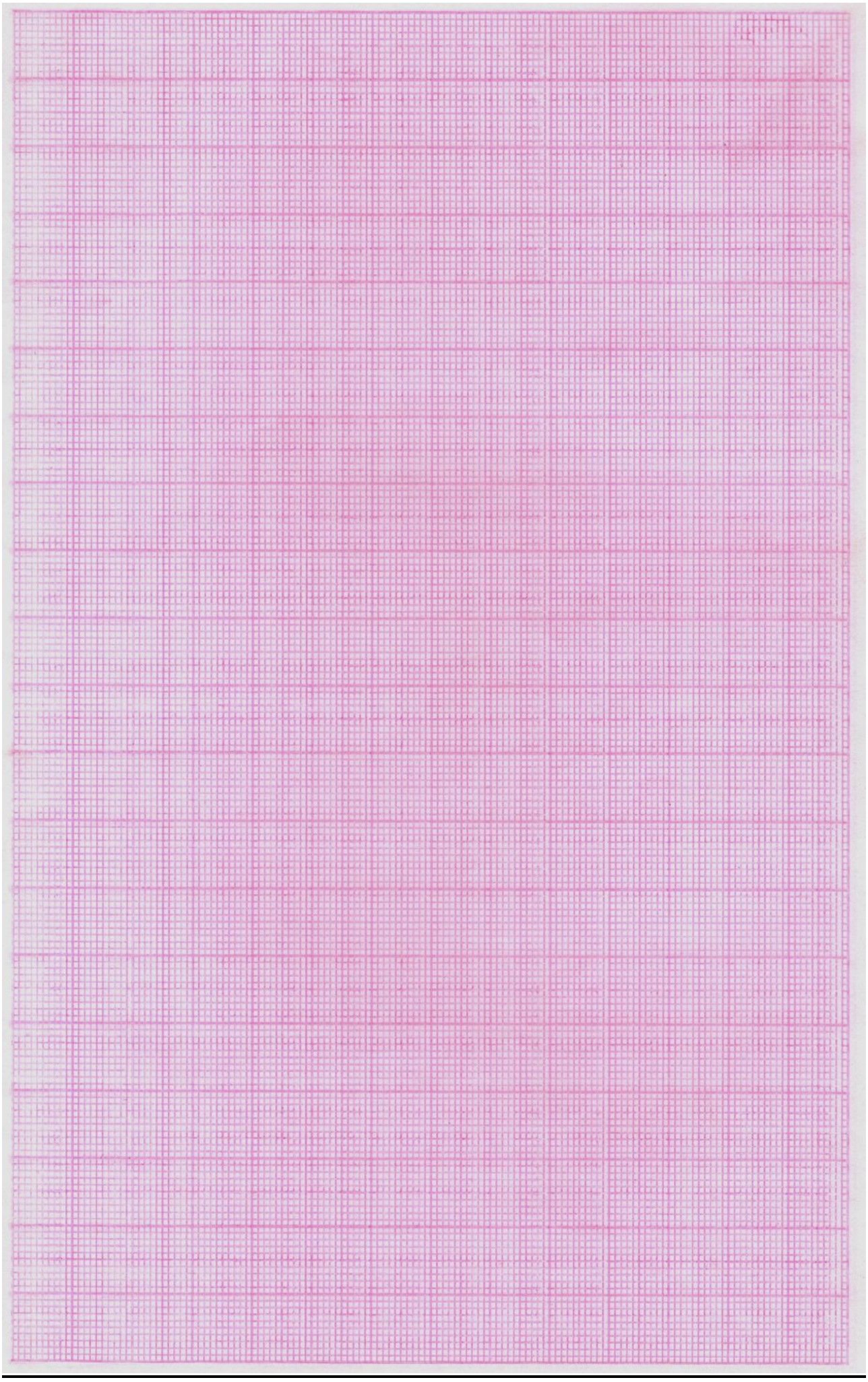
……..

……..

……..

……

.Construct the GTT curve and comment on your curve



Interpretation:

……..

……..

……..

……..

……..

Evaluation:

Name and signature of the instructor:

Date:

Good Luck