National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual-
Clinical Chemistry Department

Third Year, Semester (5)
Clinical Biochemistry -2 (MLS-CCHM-312)

Student Name: ……...

ID: ……**Batch**…….....…...

Instructions

- Wear lab coat
- Wear Gloves
- Avoid swallow any chemical
- Follow the procedures provided
- Write your results in this manual

Content

Clinical biochemistry MLS-CCH-312
Practical No (1)

Measurement of blood Urea

Enzymatic colorimetric method (Berthelot reaction)

End point

Objectives

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

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

Principle

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a blue cromophore color known as endophenol .The intensity of the color formed is proportional to the concentration of urea in the sample.

Urea + H_2O urease $2NH_3 + CO_2$

NH_4 + Salycilate+ NaCIOnitroprusside and OHEndophenol+NaCL.

REAGENT:

1- **R1 (Buffer):** Phosphate pH 6.7, EDTA, Sodium salicylate and Sodium nitroprusside. `

2- **R2 (NaCIO):** Sodium hypochlorite and Sodium hydroxide.

3- **R3 (Enzymes))**: Urease (tablets)

4- **Urea standard.** Urea 50 mg/dl. (8.3 mmol/l.) Organic matrix based primary standard.

Sample and sampling:

Serum or heparin plasma freeof hemolysis.

Procedure:

	blank	STD	Test
working reagent (R1)	2ml	2 ml	2 ml
Sample	------	-----	0.02ml
W.STD(50)mg/dl	------	0.02ml	-----

Mix well; incubate for 10 mn at R.T.

R2	2ml	2ml	2 ml

Mix and incubate for 10 mn at R.T. read the absorbance of sample and standard at 600 nm against reagent blank.

Calculation:

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

STD concentration = 50 mg/dl

……

……

……

……..

Result:

……

……

If the results are to be expressed as S.I units in mmol/l (MW. 60)

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

……..

……..

……..

……..

Reference values:

- Adults: 15----50 mg\dl.
- Urine: 20-----35 g/24hr.

Interpretation

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312
Practical No (2)
Estimation of creatinine by jaffe reaction

Objectives

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

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

Principle:

Creatinine in the sample, after removal of proteins, reacts with picric acid in alkaline media (alkaline picrate) forming red- orange chromogen (adduct) which absorbed calorimetrically at (480- 520) nm.

Reagents Composition:

10% sodium tungestate.2\3 N H2SO4.

Sodium Hydroxide 0.75 mol/L. Picric acid (saturated)

Creatinine Standard: (0.5-2mg/dl)

Sample and sampling

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/100 with D.W.

Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Procedure:

Into centrifuge tube take the followings:

1ml serum 1ml Na2WO4

1ml 2/3 H2SO4 1ml D. W

Mix well, centrifuge for 3 minutes then:

	Test	STD	Urine	Blank
Supernatant	1.5 ml	-------	--------	----------
W.STD	-------	1.5 ml	-------	-------
Diluted urine (1/100)	------	-------	1.5 ml	-------
D.W	-------	-------	-------	1.5 ml
0.75MNaOH	0.5ml	0.5ml	0.5ml	0.5ml
Picric acid	0.5ml	0.5ml	0.5ml	0.5ml

Mix well; incubate for 10 minutes at RT

Read at 490nm against reagent blank.

Calculation:

$$\frac{OD\ of\ Test}{OD\ of\ STD} \times concentration\ of\ STD \times D.F$$

STD concentration = 2 mg/dl

……

……

……

……

……

Result:

……

……..

If the results are to be expressed as S.I units in µmol/l (MW. 113 mg/dl)

$$\frac{\text{Mg/dl} \times 10 \quad \times 1000}{\text{MW}}$$

……..

……

……

Reference values:

Serum:

Male: 0.9---1.3 mg/dl. (80---115) µmol/L.

Female: 0.6----1.1mgldl (53---97) µmol/L.

Urine:

100-----200mg/dl 1------2 g/day

Interpretation

……..……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312
Practical No (3)

Estimation of creatinine by Kinetic method

With calculationof creatinine clearance

Objectives

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

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

Principle:

Creatinine in the sample reacts with alkaline picrate forming a colored complex. The complex formation rate is measured in a short period to avoid interferences.

Reagents Composition:

- Reagent (A): Sodium Hydroxide 0.4 mol/L.
- Reagent (B): Picric acid 25 mmol/L.
- Creatinine Standard: 2 mg/dl (177µmol/L).

Reagents Preparation:

Working Reagent: Mix equal volumes of reagent A and reagent B. (0.5ml reagent A + 0.5 ml reagent B)

Samples:

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/100 with D.W.

Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Procedure:

1. Pipette into a cuvette:

Working reagent	1.0 ml
STD or Sample	0.1 ml

2. Mix and insert cuvette into the photometer. Start stopwatch.

3. Record the absorbance at 490 nm after 30 second (A1) and after 90 second (A2).

Calculations:

$$\frac{sample\ (A2 - A1)}{STD\ (A2 - A1)} \times concentration\ of\ STD \times D.F$$

STD concentration = 2 mg/dl

……

……

……

Result:

……

If the results are to be expressed as S.I units in µmol/l (MW. 113 mg/dl)

$$\frac{\text{Mg/dl} \times 10 \quad \times 1000}{\text{MW}}$$

……..

……

Reference values:

Serum:

Male: 0.9---1.3 mg/dl. (80---115) µmol/L.

Female: 0.6----1.1mgldl (53---97) µmol/L.

Urine:

100-----200mg/dl

1------2 g/day

Interpretation:

…….

……..……

CREATININE CLEARANCE

Urine creatinine (mg/dl) x Urine volume (ml) = C.C (ml/ min)

Plasma creatinine (mg/dl) x Time (1440 minute)

NOTES:

TIME = 24hourx 60 minute (1440 minute)

Calculation:

……

……

……

……

Result:

……

Reference range:

Male: 97- 137 ml / min

Female: 88- 128 ml / min

Interpretation:

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (4)

Estimation of uric acid

Enzymatic method

Objectives

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

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

Principle:

The urate present in the sample react with uricase enzyme to form allantoin and H2O2.Then the H2O2 react with peroxidase enzyme in the presence of phenol and 4-amino antipyrine to form quinoneimine which has pink color read at colorimeter at 520nm. .

Reagents:

Uricase enzyme Phosphate buffer Peroxidase Phenol

4-Amino antipyrine.

Procedure:

	BLANK	TEST	STD
C.R	2.0ml	2.0ml	2.0ml
Sample	------	0.02ml	------
W.STD	--------	---------	0.02ml

Mix well; incubate at RT for 10 minutes.

Read the absorbance of test and STD against blank at 520nm.

Calculation:

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

STD concentration = 6 mg/dl

…….

…….

…….

…….

…….

Result:

…….

……..

If the results are to be expressed as S.I units in mmol/l (MW = 168.1)

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

……

……

……

……

Reference values:

In serum:

Male: 3---------7mg/dl

Female:2.5---------6.5mg/dl

Child:1.5---------4.9mg/dl.

In urine:

250------750mg/dl

Urine uric acid excretion: 7----------11g/24hour

Interpretation:

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (5)

Estimation of Bilirubin

Jendrassik and Grof method

Objectives

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

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

Principle

Bilirubin is coupled with diazotized sulfanilic acid to form azobilirubin. The color of this derivative is pH dependent, occurring as pink in acid or neutral medium and blue under alkaline conditions.

Direct (conjugated) bilirubin couples with diazotized sulfanilic acid (p-diazobenzenesulfonic acid),forming a blue color at alkaline pH.

Direct bilirubin (conjugated) + diazotized sulfanilic acid alkaline pH > blue colorazobilirubin

Indirect (unconjugated) bilirubin is diazotized only in the presence of an "accelerating" agent, caffeine-benzoate-acetate mixture. Thus, the blue azobilirubin produced in mixtures containing"accelerating" agent originates from both the **Direct** and **Indirect** fractions and reflects the **Total**bilirubin concentration.

Total bilirubin + caffeine-benzoate-acetate mixture + diazotized sulfanilicazobilirubin

Sample and sampling

Serum or heparinized plasma

Procedure:

Total (table 1) & direct (table 2)

	Reagent blank	TEST
D.W	0.1 ml	---------
Test	----------	0.1 ml
Working reagent	1.0 ml	1.0 ml

	Reagent blank	TEST
D.W	0.1 ml	--------
Test	---------	0.1 ml
Working reagent	1.0 ml	1.0 ml

- Mix well; incubate for 5 min at dark room.
- Read the absorbance of test against reagent blank at 540 nm.

Calculation:

1) For Total Bilirubin:

O.D of sample * Factor (13) = conc. Of total bilirubin

2) For Direct Bilirubin:

O.D of sample * Factor (7) = conc. Of direct bilirubin

3) For Indirect Bilirubin:

Indirect Bilirubin = Total Bilirubin _ Direct Bilirubin

Reading:

By decimal point

Result:

……

……

……

……..……..

……

……..……..

……

……..……..

……

Reference values:

1) Adults:

Total Bilirubin: Up to 1 mg/dl

Direct Bilirubin: Up to 0.25 mg/dl

Indirect Bilirubin: Up to 0.75 mg/dl

2) Newborns:

Up to 24 hr: 1------ 6 mg/dl

Up to 48 hr: 6--------- 8 mg/dl

3----- 5 days: 10-------- 15 mg/dl

Interpretation:

……

……..……..

……

……

……..……..

……

Advantage:

1) It is insensitive to sample PH changes.
2) It is insensitive to variation in protein concentration.
3) It has adequate optical sensitivity even for low bilirubin concentration.
4) It has minimal turbidity.

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (6)

Estimation of plasma total lipid

Estimation of serum cholesterol

Enzymatic method

Objectives

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

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

Principle:

Cholesterol esters hydrolyzed by cholesterol esterase to free cholesterol and fatty acids then the free cholesterol in the presence of cholesterol oxidase is oxidized to cholestene and hydrogen peroxide (H2O2) which is converted to red (pink) quinonimine in the presence of 4-aminoantipyrine and phenol, the red (pink) color produced is directly proportional to concentration of cholesterol in the sample and can be read at 520 nm.

Cholesteryl esters $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + F.F.A

Cholesterol+ O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholestenone+ H_2O_2

H_2O_2+ 4.A.A+ phenol $\xrightarrow{\text{peroxidase}}$ quinoneimine + H2O.

Sample and sampling

Serum or EDTA, Heparinised plasma (fasting if possible).

Reagents:

Cholesterol esterase Cholesterol oxidase Peroxidase, 4-aminoantipyrine

Phenol.

Procedure:

	Blank	STD	Test
Colour reagent	2.0 ml	2.0 ml	2.0 ml
Sample p/s	------	------	0.02 ml
W.STD	------	0.02	------

Mix well, incubate for 10 minute

Read at 510nm at RT.

Calculation:

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

STD concentration = 200 mg/dl

……

……

……

……

Result:

……

……

If the results are to be expressed as S.I units in mmol/l (MW. 387)

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

……..

……

……

……

Reference values:

<200 mg/dl is normal.

>220 mg/dl is at risk

Interpretation:

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical no (7)

Estimation of Serum Triglycerides

Enzymatic method

Objectives

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

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

Principle:

Triglycerides are hydrolyzed by lipase enzyme to glycerol and 3 fatty acids. The glycerol is treated by (ATP) molecules in process known as phosphorylation of glycerol to give glycerol-3-phosphate which is converted to β-hydroxy acetate+H2O2. the hydrogen peroxide react with phenol in the presence of 4.A.A in the presence of peroxidase to give red(pink) quinonimine directly proportional to conc. of triglycerides in the sample and can be read at 510nm.

Triglyceride + H2O $\xrightarrow{\text{lipase}}$ glycerol+3 fatty acids

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

Glycerol-3-phosphate $\xrightarrow{\text{G-3-PH oxidase}}$ β-hydroxy acetate+H_2O_2

H_2O_2+ 4.A.A+ phenol $\xrightarrow{\text{peroxidase}}$ quinoneimine + H2O.

Sample and sampling

Serum or EDTA, Heparinised plasma (patient must be fast overnight).

Reagents:

Lipase enzymeglycerol kinase glycerol-3-phosphate oxidase peroxidase phenol 4-aminoantipyrine

Buffer reagent.

Procedure:

	Blank	STD	Test
working reagent	2.0 ml	2.0 ml	2.0 ml
Sample p/s	------	------	0.02 ml
W.STD	------	0.02	------

Mix well, incubate for 10 mins and read at 510nm at RT.

Calculation:

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

STD concentration = 150 mg/dl

……..

……..

……..

……

Result:

……

…….

If the results are to be expressed as S.I units in mmol/l (MW. 875)

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

……

……

……

……

Reference values:

50----175 mg/dl

Interpretation:

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (8)

HDL cholesterol estimation

Precipitation method

Objectives

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

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

Principle

When serum is combined with the polyethylene glycol reagent, all betalipoproteins (LDL and VLDL) are precipitated. The HDL fraction (alphafraction) remains in the supernatant. The supernatant is then treated as a sample and assayed for cholesterol by an enzymatic method. The value obtained is the HDL cholesterol value.

Specimen:

Fresh, unhemolyzed serum is recommended.

Patient should be fasting 12-14 hours before the sample is taken.

HDL in serum is reported stable for seven days at 2-8°C and for threemonths at -20°C.9

Procedure:

Precipitation step: In test tube:

Pipette 0.5 ml (500 µl) sample into respective tubes.

Pipette 0.5 ml (500 µl) reagent into each tube and mix

Centrifuge at 1000-2000g for 10 minutes.

	Blank	STD	Test
working reagent	2.0 ml	2.0 ml	2.0 ml
Supernatant	------	------	0.02 ml
W.STD	------	0.02	------

Mix well, incubate for 10 mins and read at 510nm at RT.

Calculation:

$\frac{OD\ of\ Test}{OD\ of\ STD} \times concentration\ of\ STD \times$ D.F

Where 2 is the dilution factor.

STD concentration = 200 mg/dl

……..

……..

…….

Result:

……

……

If the results are to be expressed as S.I units in mmol/l (MW. 387)

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

……..

……

……..

Reference values:

30-75mg/dl

Interpretation:

……..

……..

LDL cholesterol equation (friedwald equation):

LDL can be calculated using the following formula:

$$\text{LDL} = \text{Total Cholesterol} - \text{HDL Cholesterol} - \frac{\text{Triglycerides}}{5}$$

……

……

……

Reference range:

66-178 mg/dl

Interpretation:

……..**……..**

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical no (9)

Estimation of sodium &pottasium

By flamephotometere

Objectives

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

1- State the theory
2- Familiarize with each part of flame photometer
3- Perform the test
4- Interpret on your results

Theory:

The purpose of the flame is twofold, chemical bonds are broken to produce atom, and then atoms absorb energy from the flame and enter an excited electronic state. The excited atoms return to the ground state by emitting light energy that is characteristic for that atomic species.

Principle:

Using compressed air, diluted serum or plasma is sprayed as affine mist of droplets (nebulizer) into anon luminous gas flame which become colored by characteristic emission of sodium or potassium metallic ions in the sample. Light of wavelength corresponding to the metal being measured which selected by alight filter or prism system and allowed to fall on photosensitive detector system.

Components:

1. Nebulizer (atomizer): In this, the sample mixed with air and sprayed to the burner at constant and reproducible rate. Compressed air is used to provide stream of air to draw in and nebulise the sample.
2. Mixing chamber with baffles: In the mixing chamber the atomized sample and fuel are mixed. The baffle deflects any large droplets as waste and allowing only the small droplets to enter the flame.
3. Burner: This converts the metallic ions to uncharged atoms and excited them to emit light.
4. Lens and Filter System: Lens focuses the emitted light from the flame and narrow band filter select the wavelength of the metal being measured (Na transmit yellow light of 589nm, K transmit at 767nm).
5. Photosensitive detector system: Is used to convert the emitted light into electric current.

Specimen:

Blood collected on heparin.

STD:

Na &K STD are prepared together from NaCl&KClas follow:

1- Na=140m.mol\l.

2- K=5m.mol\l.

Preparation of sample & STD:

Dilute both the sample, STD 1:100 AS follow:

a) Add 0.1ml from the sample to 9.9 ml of D.W.

b) Add 0.1 ml from the STD to 9.9ml of D.W.

REFRANCE VALUE:

1- Na : 135--------145 m.mol\l

K: 3.5--------5 m.mol\l.

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312
Practical No (10)

Estimation of serum calcium

(CresolphtaleinComplexone CPC)

Objectives

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

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

Principle:

The calcium present in the sample reacts with o-cresolphtalein reagent to give blue to violet color read colorimetric ally at 580nm.

REAGENT:

- **R1:** Ethanolamine 500m.mol\l
- **R2:** O-Cresolphtalein 0.62m.mol\l
  8-hydroxyquinolein 69m.mol\l
- Calcium STD: 10mg\dl

Sample and sampling

Unhaemolyzed serum or Heparinised plasma

Precaution:

1) Fasting blood sample.
2) Avoid venous stasis.
3) Don't use EDTA as anticoagulant.
4) Wash all instruments as follow: Wash by 5%HCl, then by tap water, and then by D.W (in addition the pipettes wash by the reagent).
5) Duplicate the test
6) Use glassware.

Procedure:

	Blank	STD	TEST
R1	2.0ml	2.0ml	2.0ml
R2	1drop	1drop	1drop
STD	------	0.02ml	-------
SAMPLE	------	-------	0.02ml

Mix well; incubate for 4 mins at RT.

Read at 580 nm against reagent blank.

Calculation:

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

STD concentration = 10 mg/dl

……..

……

……

Result:

……

……

If the results are to be expressed as S.I units in mmol/l (MW= 40)

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

……..

……..

……..

Reference values:

8.5--------10.5 mg\dl

Interpretation:

……

……

Calcium Correction:

Corrected Ca (m.mol\l)=$\frac{\text{40g\l —alb conc. in g\l}}{40}$ +Ca conc. (m.mol\l)

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical no (11)

Estimation of serum phosphorus

(phosphomolybdate method)

Objectives

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

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

Principle:

In organic phosphatepresent in the sample reacts with molybdic acid reagent in alkaline media (subsequent reduction) to give phosphomolybdic complex (blue) color read colorimetric ally at 710nm.

Sample and sampling

Unhaemolyzed serum or Heparinised plasma

Precausion:

- Avoid heamolysis and delay of separation.
- Avoid delay of separation of sample.
- Separate the cells from serum as soon as possible.
- Don't use EDTA as anticoagulant.
- Duplicate the test.

Reagent:

R1:molybdate borate 1.21m.mol\l.

H_2SO_4 100 mmol/l.

R2: 1'2 phenylenediamine 2.59m.mol\l

Phosphorus standard: 5mg\dl.

Reagent preparation:

Mix equal volume from R1 and R2

Procedure:

Reagent	Blank	STD	TEST
Working reagent	3 ml	3 ml	3 ml
STD	----------	0.1ml	-------
SAMPLE	----------	-------	0.1ml

Mix well; incubate for 30 mins at RT.

Read at 710 nm.

Calculation:

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

STD concentration = 10 mg/dl

……

……

……

Result:

……

……

If the results are to be expressed as S.I units in mmol/l (MW= 35)

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

……

……

……

Referance value:

In serum:

(2.5--------5) mg\dl (old unit)

(0.8--------1.8)mmol\l (SIU) for adults.

(4---------7) mg/dl.

1.3-------2.2 mmol/l. for children.

Interpretation:

……..

……..

.....

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (12)

Estimation of C. S. F. glucose &protein

Objectives

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

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

- **C.S.F glucose:**

It can be estimated by glucose oxidase method.

(Like estimation of blood glucose)

Reagent	Blank	STD	Test
Glucose reagent	2 ml	2 ml	2 ml
C.S.F	------	------	0.02 ml
W.STD	-------	0.02 ml	-------

Mix well; incubate for 10 mins at RT

Read at 520 nm**.**

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)

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

……

……

……

Reference value:

(40-------80) mg\dl.

Interpretation:

……

……

1) C.S.F protien:

It can be estimated by using 3%T.C.A or diluted S.S.A with Na_2CuSO_4.

Procedure:

	BLANK	STD	TEST
3%T.C.A	2ml	2ml	2ml
Sample	**------**	**------**	0.5ml
W.STD(50mg\dl)	**-------**	0.5ml	**------**

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

Read at filter 430nm.

Calculation:

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

STD concentration = 50 mg/dl

……

……

……..

Result:

……

……

Reference range:

15------45 mg/dl

Interpretation:

……..…

……..

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312
Practical (13)

Estimation of enzymes activity

Alkaline phosphatase (ALP)

Kinetic method

Objectives

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

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

Principle:

ALP catalyze the hydrolysis of 4-NPP (4-nitrophenylphosphate) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

p- nitrophenyle phosphate + H_2O $\xrightarrow{ALP}$ Nitrophenol + Inorganic phosphate

Assay:

By spectrophotometer

Sample and sampling

Serum or heparanized plasma, free of hemolysis.

Other anticoagulants such as EDTA, oxalate and citrate inhibit the enzyme.

ALP in serum or plasma is stable for 7 days at 2-8C.

Reagent composition:

R1: ALP buffer DEA buffer 1.25 mol/L PH 10.2, MgCl 0.6m.mol/L.

R2: ALP substrate (4-NPP) 50 m.mol/L.

Reagent preparation:

Working reagent: Mix 4 ml of R1 + 1ml of R2.

Procedure:

1) Set the photometer to 0 absorbance with D.W.
2) Pipette into a cuvette the following:

Working reagent	1.0 ml
Sample	0.02 ml

3) Mix gently. Insert cuvette into cell holder and start stopwatch.
4) Incubate for 1 minute and record initial absorbance reading.
5) Repeat the absorbance readings exactly after I, 2, and 3 minutes.
6) Calculate the difference between absorbances.

7) Calculate the mean of the results to obtain the average change in absorbance per minutes (▲A/ min).

Calculations:

U/L = ▲A x 2764

R1=…….. R2=…….

R3=…….

R2-R1=…… R3-R2=……

$$\Delta \text{ Ab/ min} = \frac{(R3-R2) + (R2-R1)}{2}$$

…….

……

Result

……..

……..

If results are to be expressed as SI units apply:

U/Lx 0.01667 = µkat/L.

……..

……..

……

Referance value:

	25°C
Children up to	480U/L (8.0 µkat/L).
Adults up to	180 U/L (3.0 µkat/L).

	30°C
Children up to	580U/L (9.6 µkat/L).
Adults up to	220 U/L (3.7 µkat/L).

	37°C
Children up to	800U/L (13.3 µkat/L).
Adults up to	270 U/L (4.5 µkat/L).

Interpretation:

……..

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312
Practical No (14)
Estimation of AST
Kinetic method

Objectives

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

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

Principle

Kinetic determination of AST activity based upon the following reaction

L-aspartate + α Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L- Glutamate

Oxaloacetate +NADH +H^+ $\xrightarrow{MDH}$ L- malate +NAD^+

- AST: Aspartate aminotransferase
- MDH: Malate dehydrogenase

Procedure

Working reagent 1000µl

Sample 100µl

Mix and measure the change in absorbance per minute during 3 minutes

Calculation

ALP activity = Δ(OD/min) x 1745

R1=…….. R2=…….

R3=…….

R2-R1=……R3-R2=……

$$\Delta \text{ Ab/ min} = \frac{(R3-R2) + (R2-R1)}{2}$$

……

……..

Result

……..

Reference range

Up to 40 U/l

Interpretation

……

……

Evaluation:

Name and signature of the instructor:

Date:

Clinical biochemistry MLS-CCH-312

Practical No (15)

Estimation of AlT activity

Kinetic method

Objectives

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

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

Principle

Kinetic determination of AlT activity based upon the following reaction

L-alanine + α Ketoglutarate $\xrightarrow{\text{AlT}}$ pyruvate + L- Glutamate

pyruvate +NADH +H^+ $\xrightarrow{\text{LDH}}$ L- lactate +NAD^+

- ALT: Alanine aminotransferase
- LDH: lactate dehydrogenase

Procedure

Working reagent 1000µl

Sample 100µl

Mix and measure the change in absorbance per minute during 3 minutes

Calculation

ALP activity = Δ(OD/min) x 1745

R1=…….. R2=…….

R3=…….

R2-R1=……R3-R2=……

$$\Delta \text{ Ab/ min} = \frac{(R3-R2) + (R2-R1)}{2}$$

……
……
……
……

Result

……

……

Reference range

Up to 35 U/l

Interpretation

……..

……

Evaluation:

Name and signature of the instructor:

Date:

GOOD LUCK