**APPENDIX**

**Glucose assay**

**Assay principle**

The principles outcome of glucose is based on the principle of competitive binding between glucose in the test specimen and GOD-PAP reagent of glucose. The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4- aminophenazone to a red-violet quinoneimine dye as indicator.

**Reaction**

GOD

Glucose + O2 + H2O Gluconic acid + H2O2

POD

2H2O2 + 4-aminophenazone + Phenol Quinoneinine + 4 H2O

**Materials and reagents**

1. Serum sample

2. Glucose conjugate reagent

3. Precision pipettes 10 µl, 1.0 ml

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

The sterile eppendorf tubes were taken. 1000 μl of Glucose conjugate reagent was taken each into each eppendorf tube. Then 10μl of Glucose standard was added in with the reagent in eppendorf tube and 10μl of samples serum were taken in each sample eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Glucose standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Glucose conjugate reagent was examined by Biochemical analyzer and the reading was taken. The standard value was used as a compared tool.

**Total protein assay**

**Assay principle**

The principle outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

**Materials and reagents**

1. Serum sample

2. Total protein conjugate reagent

3. Precision pipettes: 20μl and 1.0ml

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20μl of total protein standards was taken in an eppendorf tube and 20μl of sample serums were taken in each 24 eppendorf tube. 1000μl of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Total protein standards with conjugate.

**Reaction**

**Albumin assay**

**Assay principle**

The principles outcome of albumin is based on the principle of competitive bindings between albumin and albumin reagent. Bromocresol green forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentration in the sample.

**Materials and reagents**

1. Serum sample

2. Albumin conjugate reagent

3. Precision pipettes

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This was a photometric colorimetric test for albumin is called Bromo Cresol Green method. The sterile eppendorf tubes were taken. Then 10μl of albumin standards was taken in an eppendorf tube and 10μl of sample serum were taken in each eppendorf tube. 1000μl of albumin conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Albumin standards with conjugate reagent were examined first for determined of the standard value. Then all 100 eppendorf tubes containing sample serum with albumin conjugate reagent was examined using automated humalyzer and the reading was taken. The standard value was used as a compared tool.

**Cholesterol assay**

**Assay principle**

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

**Reaction**

Cholesterol esterage

Cholesterol ester +H2O Cholesterol +Fatty acid

Cholesterol oxidase

Cholesterol+O2 Cholesterol-3-one+H2O2

Peroxidase

2H2O2+Phenol+4-Aminoantipyrine quinoneimine+4H2O

**Materials and reagents**

1. Serum sample

2. Cholesterol conjugate reagent

3. Precision pipettes

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This was an enzmatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tube was taken. Then 10μl of cholesterol standards was taken in an eppendorf tube and 10μl of sample serums were taken in each eppendorf tube. 1000μl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

**Triglyceride**

**Assay Principle**

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4 –aminophenezone and 4 –Chlorophenol under the catalytic influences of peroxidease.

**Reaction**

Lipases

Triglycerides + H2O Glycerol+Fatty acid

 GK

Glycerol +ATP glycerol 3 phosphate +ADP

GPO

Glycerol -3- phosphate +O2 Didydroxyacetone+Phosphate+H2O2

POD

2H2O+4aminophenazone +4 Chlohenolquiniamine Quiniamine + HCl+4H2O

**Materials and reagent**

1. Serum sample

2. TG conjugate reagent

3. Precision pipettes

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves

**Procedure**

The sterile eppendorf tubes were taken. Then 1000μl TG standards was taken in an eppendorf tube and 10μl of sample serums were taken in each eppendorf tube The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

**LDL assay**

**Assay Principle**

The principles outcome of LDL is based on the principle of competitive bindings between LDL and LDL reagent. Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (PH-5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant. The absorbance of this complex is proportional to the LDL concentration in the sample.

**Materials and reagents**

1. Serum sample

2. LDL conjugate reagent

3. Precision pipettes

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

The sterile eppendorf tubes were taken. Then 100μl of LDL standards was taken in an eppendorf tube and 100μl of sample serums were taken in each eppendorf tube. 1000μl of LDL conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. The LDL concentration of the supernatant was determined within 1 hour after centrifugation. LDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with LDL conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

**HDL assay**

**Assay Principle**

Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitavily by the addition of phosphotangstic acid in the presence of magnesium ions.

After centrifugation, the cholesterol concentration in the HDL (high density Lipoprotein) fraction, which remains in the supernatant, is determined.

**Materials and reagents**

1. Serum sample

2. HDL conjugate reagent

3. Precision pipettes

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol.

**Procedure**

The sterile eppendorf tubes were taken. Then 400μl of HDL standards was taken in an eppendorf tube and 200μl of sample serums were taken in each eppendorf tube. 100μl of distilled water was then added to each eppendorf tube. The eppendorf tube was kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. Then 50 μl HDL concentration of the supernatant was taken and 1000 μl Cholesterol reagent added determined within 1 hour after centrifugation. HDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with HDL conjugate reagent was examined by automated humalyzer and the r reading was taken. The standard value was used as a compared tool, absorbent paper or paper towel or cotton and gloves.