MATERIALS AND METHODS

The present study screened 851 patients out of which 320 subjects were included in the study, comprising of both the normal subjects and the patients suffering from thyroid disease.

SPECIMEN CCLLECTION AND STORAGE

Serum is the preferred specimen for the measurement of T_4; plasma with ethylene diamine tetra-acetic acid (EDTA) or heparin as anticoagulant may be used. However, plasma tends to form fibrin clots after freezing and thawing; these clots may interfere mechanically with an assay, especially an automated system. Gel barrier tubes have no adverse effect on T_4 concentration. T_4 in serum is quite stable, storage of serum specimen at room temperature upto 7 days results in no appreciable loss of T_4. However, serum specimens are best stored at 2 to 8°C if they will not be tested within 24 hours. If longer periods of storage are necessary, freezing the specimens is recommended. Frozen specimens are stable for at least 30 days.

Patients undergoing therapy for a thyroid disorder should stop treatment 1 month before sampling if a true baseline is to be established (Tietz p. 1514, 3rd edition).

The study of normal subjects comprised of 30 persons, consisting of 26 females and four males, between the age of seven to sixty-five years. These persons were selected from the S. S. G. Hospital and Medical College, Baroda.

The patients were divided into eleven groups as shown below:

Group	Patients suffering from	Total No.	Females	Males	Range of age (in yrs)
I	Hyperthyroidism	30	18	12	18-68
II	T_3 Toxicosis	30	23	07	32-75
III	Hyperthyroid Follow up cases	30	18	12	18-60
IV	Hyperthyroid with Over-treatment	20	14	06	18-68
V	Hypothyroidism with hypercholesterolemia	30	23	07	24-60
VI	Hypothyroidism with hyperlipoproteinamia type IIb	30	22	08	15-70
VII	Severe hypothyroidism with hyperlipoproteinemia type III	30	21	09	13-80
VIII	Hypothyroid Follow up cases	30	24	06	20-80
IX	Hypothyroid with Over-treatment	20	15	05	20-80
X	Thyroid disorder secondary to liver disease	20	18	02	28-48
XI	Thyroid disorder secondary to renal disease	20	13	07	20-65

All these patients were selected from the S. S. G. Hospital, Baroda.

Blood was collected in plain bulb after an overnight fast of 12 hours, and the serum was separated immediately by centrifugation and preserved at 0-4°C.

The samples from all the subjects were analyzed immediately for [1] Thyrotropin (TSH) [2] Tri-iodothyronine (T_3) [3] Thyroxine (T_4) [4] Triglyceride (TG) [5] Cholesterol (TC) [6] High Density Lipoprotein (HDL) [7] sodium (Na) [8] Potassium (K) [9] Lactate dehydrogenase (LDH) [10] Serum Aspartate Transaminase (AST) [11] Low Density Lipoprotein (LDL) and [12] Very Low Density Lipoprotein (VLDL). VLDL and LDL was calculated by Friedewald formula.

All the methods selected for the present study are standard procedures, giving reproducible results and the kits used are from well established companies like ACCUREX, ERBA, ECOLINE and Herichson Diagnostics. All the results obtained are depending upon the facilities of instruments and apparatus available in our Government Medical College.

SERUM CHOLESTEROL

Principle

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction, cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxidase oxidatively couples with 4-amino-antipyrine and phenol to produce red quinone-imine dyes which has absorbance maximum at 510 nm (505-530 nm). The intensity of the red colour is proportional to the amount of total cholesterol in the specimen.

$$\text{Cholesterol esters} \xrightarrow{\text{CHE*}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHO*}} H_2O_2 + \text{Cholest-4-en-3-one}$$

$$2H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{POD*}} \text{Red Quinoneimine Dye} + H_2O + \text{Phenol}$$

*Abbreviations

CHE = Cholesterol Esterase

CHO = Cholesterol Oxidase

POD = Peroxidase

Expected Values

Desirable cholesterol	< 200 mg/dl
Borderline cholesterol	200-239 mg/dl
High cholesterol	> 240 mg/dl

TRIGLYCERIDE

Glycerol released from hydrolysis of triglycerides by lipoprotein lipase was converted by glycerol kinase into glycerol-3-phosphate which oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red coloured compound.

$$\text{Triglyceride} \xrightarrow{\text{LPL*}} \text{Fatty acids + Glycerol}$$

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

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO*}} \text{Dihydroxy acetone phosphate + } H_2O_2$$

$$H_2O_2 + \text{Phenolic chromogens} \xrightarrow{\text{POD*}} \text{Red colour}$$

*Abbreviations

LPL = Lipoprotein Lipase

GK = Glycerol Kinase

GPO = Glycerol Phosphate Oxidase

POD = Peroxidase

Expected values : Upto 170 mg%

HDL CHOLESTEROL

Principle

Phosphotungstate / Mg^{+2} precipitates chylomicrons, LDL and VLDL fractions. High density lipoprotein (HDL) fraction remains unaffected in supernatant. Cholesterol content of HDL fraction is assayed using AutoZyme Cholesterol.

$$\text{Serum/Plasma} \xrightarrow[Mg^{+2}]{\text{Phosphotungstate}} \underset{\text{(Supernatant)}}{\text{HDL fraction}} + \underset{\text{(precipitate)}}{(\text{LDL + VLDL + Chylomicron})}$$

Expected Values : 30-60 mg% HDL-Cholesterol.

T_4 Thyroxine

Principle

The procedure follows the basic principle of enzyme immunoassay where there is competition between an unlabelled antigen and an enzyme-labelled antigen for a fixed number of antibody binding sites. The amount of enzyme-labelled antigen bound to the antibody is inversely proportional to the concentration of the unlabelled analyte present. Unbound materials are removed by decanting and washing the wells. The absorbance measured is inversely proportional to the concentration of T_4 present in the serum. A set of T_4 standards is used to plot a standard curve of absorbance versus T_4 concentration from which the T_4 concentration in the unknowns can be calculated.

Normal Range : 5 to 13 μg/dl

Total T_3 (Herickson Kits)

Principle

The procedure follows the basic principle of enzyme immunoassay where there is competition between an unlabelled antigen and an enzyme-labelled antigen for a fixed number of antibody binding sites. The amount of enzyme-labelled antigen bound to the antibody is inversely proportional to the concentration of the unlabelled analyte present. Unbound materials are removed by decanting

and washing the well. The absorbance measured is inversely proportional to the concentration of T_3 present in the serum. A set of T_3 standards is used to plot a standard curve of absorbance.

Expected Value 0.75 to 2 ng/ml

TSH (Herickson Kits)

The TSH-ELIA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase (microtitre wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horse raddish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is stopped with the addition of 2 NHCl, and the colour is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the colour intensity of the test sample.

Expected Value : 0.4-7 μIU/ml

LDH (Ecoline Kits)

Principle

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}$$

Decrease in absorbance due to oxidation of NADH is monitored at 340 nm and is directly proportional to LDH activity.

Normal Range at 37°C : 225-450 U/L

AST (GOT) (Accurex Kits)

Principle

$$\text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{GOT (AST)}} \text{Oxaloacetate} + \text{L-Glutamate}$$

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

The conversion of NADH to NAD^+ is proportional to the concentration of GOT in serum; and is measured at 340 nm as rate of decrease in absorbance.

Normal Range : Upto 40 IU/L at 37°C.

SODIUM-POTASSIUM MEASUREMENT

Using ION SELECTIVE ELECTRODE BY AVL INSTRUMENT

Normal Range : Na^+ = 134-146 mmol/L

K^+ = 3.6-5 mmol/L

MATERIALS AND METHODS FOR BACTERIAL WORK

The protein was estimated by the method of Lowry et al (1951).

Composition of minimal medium M9 used is as follows for 100 ml:

1. Na_2HPO_4 → 0.6 gm
2. KH_2PO_4 → 0.3 gm
3. NaCl → 50 mg
4. NH_4Cl → 100 mg
5. D. W.(Distilled H_2O) → 95 ml

Autoclave the contents

6. 1 M $MgSO_4$ → 0.2 ml
7. 20% Glucose → 2 ml
8. 1 M $CaCl_2$ → 0.02 ml