ANNEXURE - 1

KPU OF PRACTITIONERS

(Key Informant Interviews)

Questions related to the Knowledge pertaining to Use of *Aegle Marmelos (L.) Correa* in the treatment of various diseases

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia resulting from defects in insulin secetion, insulin action or both. Diabetes is the most common form of diabetes. In recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies are prescribed by Ayurvedic, Unani , Naturopathy and other indigenous systems of medicine are commonly used in India. *Aegle Marmelos (L.) Correa* (Bael) is indigenous to India and is one of the most useful medicinal plant of India because all parts of the tree (stem, root, bark,leaves and fruits) have medicinal virtues and have a long tradition as herbal medicine.

The aim of this research is to study the impact of *Aegle Marmelos (L.) Correa* leaf extract on the glycemic and lipidemic profile, liver and kidney functions of the Type II diabetic subjects. As part of the Phase II study, KPU of total (n=30) Alternative medicine practitioners will be done on the basis of Key informant interviews. Out of total 30 doctors or practitioners in the field of Ayurvedic, Unani and Naturopathy, 10 practitioners from each i.e. Ayurved, 10 Unani and 10 Naturopathy will be interviewed to know their KPU.

Name of the Practitioner:_____

Field of Practice-Ayurved / Naturopathy :_____

Address or venue of Practice: _____

Open-ended questions:

Q.1 Do you know about medicinal properties of *Aegle Marmelos (L.) Correa* (Bael) plant?

_____

Q.2 Which parts of bael is most beneficial in the treatment of various diseases?

_____

Q.3 Do you use bael in the treatment of Diabetes specially type-2?

_____

Q.4 Which part of the bael is most beneficial in the treatment of Diabetes?

_____

Q.5 What is the form in which bael is being used in the treatment ?

_____

Q.6 Is bael given in raw or powdered or decoction form?

_____

Q.7 Which form is most acceptable to the patients?

_____

Q.8 What is the dosage of bael given to the patients in various diseases specially diabetes?

_____

Q.9 What is the duration of the treatment given to the patients?

_____

Q.10 Acceptability of the bael by the patients- Whether the leaf or leaf juice or powder is astringent, bitter or normal?

_____

Q.11 From where you procure the bael plant or leaves?

_____

Q.12 Is bael also used in poly herbal formulations?

_____

ANNEXURE - 2

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 – INDIA

Estb 1949

Sensory evaluation sheet for dosage selection of Aegle Marmelos (L.) Corrrea leaf juice

Name: _____ FBS_____
Date:_____ Sign_____
Address:_____

Evaluate these coded samples on a 9 point hedonic scale ranging from "Like extremely" to "Dislike extremely". Write your comments.

Score	Grade	Sample A	Sample B	Sample C
1	Dislike extremely			
2	Dislike very much			
3	Dislike moderately			
4	Dislike slightly			
5	Neither like nor dislike			
6	Like slightly			
7	Like moderately			
8	Like very much			
9	Like extremely			

Comments:

_____

_____

ANNEXURE - 3

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 - INDIA

Estb 1949

CONSENT FORM FOR CLINICAL TRIAL

Dated:_____

INDEPTH STUDY OF THE ANTIOXIDANT PROFILE OF *AEGLE MARMELOS (L.) CORREA* AND ITS IMPACT ON BLOOD SUGAR LEVELS OF TYPE II DIABETES MELLITUS SUBJECTS

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Type-2 diabetes mellitus is the most common form of diabetes mellitus.

Currently available therapeutic options for type-2 diabetes, such as dietary modifications, oral hypoglycemic and insulin have limitations of their own. Therefore the search for more effective and safer anti-hyperglycemic agents has become an area of active research. In recent years the popularity of complementary medicine has increased. The World Health Organization (WHO) has also recommended the evaluation of the effectiveness of plants traditionally used for the treatment of various conditions where we lack safe modern drugs.

Aegle Marmelos (L.) Correa commonly known as bael is indegenous to India and is one of the most useful medicinal plants of India. Recent studies are confirming hypoglycaemic effect of this plant leaf in clinical trials on animals. No such findings have been reported on human subjects.

The aim of this research is to study the impact of *Aegle Marmelos (L.) Correa* leaf juice on the glycemic and lipidemic profile, liver and kidney functions of the diabetic mellitus type-2 patients. The patients will be assessed for various parameters like HBA1c, FBS, Hs-CRP, SGOT, SGPT, Creatinine and Total proteins by drawing their blood samples. The confidentiality of the subjects will be maintained including their personal identity and personal medical record. The testing of parameters will bear no financial load to the any participants during the entire intervention programme. They will be made aware of the information limited to their participation during the entire course of the study.

Vinita Nigam
Ph.D Scholar
Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda

I have been informed regarding the importance of an intervention programme including the benefits involved regarding health- _____
I am fully aware of my rights regarding safety and well-being -_____
I will co-operate fully as regards diet supplementation and medical testing-_____
I am willingly participating in the study_____
I am willing to provide data on medical history, Nutritional status, 6-10 ml blood for biochemicalprofile (Hb, HbA1c, Blood glucose levels, lipid profile, liver and kidney function) and dietary profile._____
I agree to be a part of this study titled "**Indepth Study of The Antioxidant Profile of *Aegle Marmelos (L.) Correa* And Its Impact onType II Diabetes Mellitus**" for the duration of three months.
Name of the participant:-_____
Address:-_____
Date:-_____

Sign:-_____

ANNEXURE - 4

DIABETES CARE PROFILE QUESTIONNAIRE

Form Number -------
Date ----------

Name of the investigator ----------
Subject's name----------

Section I - Demographics

Please answer each of the following questions by filling in the blanks with the correct answers or by choosing the single best answer.

Q1. Age: __ __ years old

Q2. Birth date: __ __ /__ __ /__ __
(Month / Day /Year)

Q3 Sex: ☐1 Male ☐2 Female

Q4. In which year was your diabetes identified? (Please enter the year) __ __ __ __

Q5. What is your marital status? (check one box)

	Never married		Separated / Divorced
	Married		Widowed

☐1 Never married
☐2 Married
☐3 Separated/Divorced
☐4 Widowed

Q6. What is your ethnic origin/religion? (Check one box)

Hindu	Muslim	Sikh
Parsi	Christian	Other-

Q7. Where do you live most of the year? (Check one box)
Address: _____

Q8. How many family members you have? ----------

Q10. How much education have you had?
(Check one box)

Less than VII std.		Upto X std. or less		Upto XII std. or less	Any other specify
Graduation		Post-graduation		Technical degree	

Q11. Which of the following best describes your current employment status? (check one box)

Working full time	Working part time	Unemployed	Home maker
In school	Retired	Disabled	Something else (specify)

12. Do you test your blood sugar? (check one box)
☐1 No ☐2 Yes ⟶ Q14a. How many days a week do you test yourbloodSugar?

↓ ___ (days / week) ↓

Q14c. Do you keep a record of your blood sugar test results? (Check one box)

☐1 No ☐2 Yes☐3Only Unusual Values

Section II – Health Status

general, would you say your health is: (check one box)

☐1	☐2	☐3	☐4	☐5
Excellent	Very Good	Good	Fair	Poor

Q2.These questions ask about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the Way you have been feeling.

How much of the time **during the past 4 weeks**: (circle one answer for each line)

	All of the Time	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
A. Have you felt **calm and peaceful**?	1	2	3	4	5	6
B. Did you have a **lot of energy**?	1	2	3	4	5	6
C. Have you felt **downhearted and angry**?	1	2	3	4	5	6

Section III - Understanding of overall diabetes care

Q1. How do you rate your understanding of: (circle one answer for each line)	**Poor**		**Good**		**Excellent**
a) overall diabetes care	1	2	3	4	5
b) coping with stress	1	2	3	4	5
c) diet for blood sugar control	1	2	3	4	5
d) the role of exercise in diabetes care	1	2	3	4	5
e) medications you are taking	1	2	3	4	5
f) how to use the results of blood sugar monitoring	1	2	3	4	5
g) how diet, exercise, and medicines affect blood sugar levels	1	2	3	4	5
h) prevention and treatment of high blood sugar	1	2	3	4	5
i) prevention and treatment of low blood sugar	1	2	3	4	5
j) prevention of long-term complications of diabetes	1	2	3	4	5
k) foot care	1	2	3	4	5
l) benefits of improving blood sugar control	1	2	3	4	5

Section IV - Understanding of blood sugar

Q1.How many **times** in the last **month** have you had a **low blood sugar** (glucose) reaction with symptoms such as sweating, weakness, anxiety, trembling, hunger or headache?

☐$_1$ 0 times

☐$_2$ 1-3 times

☐$_3$ 4-6 times or more

Q2.How many **days** in the last **month** have you had **high blood sugar** with symptoms such as thirst, dry mouth and skin, increased sugar in the urine, less appetite, nausea, or fatigue?

☐$_1$ 0 days

☐$_2$ 1-3 days

☐$_3$ 4-6 days or more

Q3.Have you been diagnosed with hepatic or renal dysfunction ?

Yes-_____No-_____

Section V - Monitoring parameters and Understanding

Q1. How many days a week have you been told to test:

a) urine sugar? _____ (days per week) ☐$_9$ Not told to test

b) blood sugar? _____ (days per week) ☐$_9$ Not told to test

If you **do not** test for sugar, skip Question No. 2.

Q3. Have you ever received diabetes education? ☐$_1$ No ☐$_2$ Yes

If No, skip Question No. 4

For the following questions, please circle the appropriate response.

(circle one answer for each line)

Q 4. How do you rate your understanding of:	**Poor**		**Good**		***Excellent***
a) diet and blood sugar control	1	2	3	4	5
b) weight management	1	2	3	4	5
c) exercise	1	2	3	4	5
d) use of insulin/pills	1	2	3	4	5
e) sugar testing	1	2	3	4	5
f) foot care	1	2	3	4	5
g) complications of diabetes	1	2	3	4	5
h) eye care	1	2	3	4	5
i) combining diabetes medication with other medications	1	2	3	4	5
j) alcohol use and diabetes	1	2	3	4	5

Section VI- Physical Activities

Q1.The following questions are about activities you might do during a day. (Circle one answer on each line)

		Yes, Limited A Lot	Yes, Limited A Little	No, Not Limited At All
A.	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports?	1	2	3
B.	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1	2	3
C.	**Lifting or carrying groceries?**	1	2	3
D.	Climbing several flights of stairs?	1	2	3
E.	Climbing one flight of stairs?	1	2	3
F.	Bending, kneeling, or stooping?	1	2	3
G.	Walking more than a mile?	1	2	3
H.	Walking several blocks?	1	2	3
I.	Walking one block?	1	2	3
J.	Bathing or dressing yourself?	1	2	3

Q13. Have you ever filled out this form before? (Check one box)

☐1 Yes
☐2 No
☐3 Don't remember

Section: Impact of the treatment

Data showing impact of the treatment on various anthropometric measurements

Group	Height (cms)	Body weight(Kg) Initial Final	Body mass (m^2) Initial Final	BMI (Kg/m^2) Initial Final
Control				
Treatment A				

Data showing impact of the treatment on various medical parameters

Group	HbA1C Baseline Final	Fasting blood glucose (mg/dl) Baseline Final	Total Cholesterol (%) Baseline Final
Control			
Treatment			

Group	SGOT Baseline Final	SGPT Baseline Final	Creatinine Baseline Final	Total Proteins (%) Baseline Final
Control				
Treatment				

ANNEXURE - 5

PHASE III- CLINICAL TRIAL -GENERAL INFORMATION AND MEDICAL HISTORY

GENERAL INFORMATION: CODE:

1. Socio-Economic Status

Name of the respondent: _____ Address: _____

Age: _____

Gender: Female ☐ Male ☐

Religion: Hindu ☐ Muslim ☐

Christian ☐ Other ☐

Educational qualification

Type of family: Nuclear ☐ Joint ☐ Extended ☐

No. of family members: _____

Family income (monthly/ annual): _____

2. Addiction Pattern:

SR. No.	Addiction	Yes	No	Age of initiation	Frequency
1	Alcohol				
2	Cigarette				
3	Tobacco powder				

3. Family History:

SR. No.	Father	Mother	Siblings

4. Morbidity Profile

1) Problems of oral cavity:	
a) Mouth ulcers	
b) Inflammation of tongue	
c) Dental caries	
2) Problems of gastrointestinal tract:	
a) Gastritis	
b) Ulcerative colitis	

c) Dysentery	
3) Problems of respiratory tract:	
a) Pneumonia	
b) Asthma	
c) Recurrent cold	
d) Tonsilitis	
4) Problems of hepatobiliary tract	
a) Jaundice	
b) Any other-specify:	

5. Anthropometric Measurements :

Parameter	Value
Weight (Kg)	
BMI	
Body composition	

6. Clinical Signs And Symptoms:

Area of examination	**Sign**	**Yes/no**
Eyes	Pale conjunctiva	
	Eyelid light pink	
Lips	Angular stomatitis	
Nails	Spoon shaped nails	
Other	Fatigue	
	Breathlessness	
	Palpitations	
	Dizziness	
	Headache	
	Insomnia	
	Numbness in fingers and toes	

24 HOUR RECALL: Code:

TIME	MEAL	INGREDIENTS	RAW ITEM		COOKED ITEM	
			Amount (g)	Volume (ml)	Amount (g)	Volume (ml)
	BREAKFAST					
	LUNCH					
	SNACKS					
	DINNER					

FOOD FREQUENCY QUESTIONNAIRE:

Food frequency:

Name: _____
Code:_____

Date:

Antioxidant and Iron sources:

Food	Daily	Once a week	Once in 15 days	Once a month	occasionally	Never
CEREALS: • Rajgeera • Bajra						
WHOLE FRUITS: • Citrus • Lemon • Berries • Others FRUIT JUICES						
VEGETABLES: • GLV • Others • Vegetable soup • Salads • Spices • Rhizomes						
MILK AND MILK PRODUCTS:						
OILSEEDS: • Flaxseeds • Garden cress seeds • Niger seeds						
MEAT, FISH AND POULTRY:						
TEA/COFFEE:						
CARBONATED BEVERAGES:						

ANNEXURE - 6

Details of Bio-chemical Parameters

Estimation of FBS and PP2BS-

FBS and PP2BS was estimated using enzymatic reference method ((Trinder P., 1969) using enzymatic GOD-POD Method.

Test Principal: GOD

Glucose+ O_2+ H_2O ⟶ Gluconate + H_2O_2

POD ⟶

$2H_2O2$+ 4 - aminophenazone + Phenol 4-(p-benzoquinone-mono-imino) Phenazone + 4 H_2O

In the determination of phosphate, use is made of the fact that phenol in the presence of an oxidizing reagent gives a purple colour with 4 amino phenazone. The possibility that the H_2O_2 released in the reaction of glucose oxidase with glucose could act as oxidizing agent was investigated and it was found that the system of phenol and 4-amino phenazone is well suited to the determination of the glucose. By suitable adjustment of conditions the colour develops completely in 10 minutes, being stable thereafter for at least 30 minutes. Using a single-solution photophosphotungstic acid precipitant containing phenol to precipitate blood protein the only other solution required is the one containing glucose-oxidase, peroxidase and 4-amino phenazone. These solution contain azide as preservative; azide has no effect on the rate of color development. In the macro and micro automated methods, the two solutions required are a diluents containing 4 amino phenazone and a colour reagent glucose oxidase, peroxidase and a phenol.

Reference Ranges-

Normal Range Fasting blood sugar- 70-110 mg/dl
Post prandial blood sugar- 110-140 mg/dl

Estimation of HbA1c-

Principle

It works on the principle of high performance liquid chromatography (HPLC), which is considered the "Gold Standard" technology in the follow-up of the plasma glucose concentration of diabetic patients over time, via the measurement of HbA1c (glycated hemoglobin fraction).

It is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column and a detector that shows the retention time of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed and the solvent(s) used. Cation exchange columns employ the differences in ionic interactions between hemoglobin components to separate them in a span of 1.6 min. A step gradient elution is used to separate HbF, s-A1c, total A1 and Hb varient with three types of G8 varient elution buffer His (G8 varint elution buffer His no. 1, 2, and 3(S)) of different salt concentrations.

Refernce Range (Normal Values)

1. Below 6% Normal
2. 6%-7% Good Control
3. 7%-8% Fair control
4. 8%-10% Unsatisfactory Control
5. > 10% Poor Control

Estimation of Total Cholesterol

The total cholesterol was estimated using the CHOD POD method with the help of enzymatic kits. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to free cholesterol to free fatty acids. This free cholesterol is then oxidized by cholesterol oxidase (CHOD) to cholest-4-ene-3-one & Hydrogen Peroxide (H202). The liberated H_20_2 then combines with phenol & 4-aminoantipyrine to produce quinonimine, a red colored complex in presence of peroxidase (POD). The color of which is directly proportional to the quantity of cholesterol present in the serum. This is measured calorimetrically by automated chemistry analyzers at 500 nm. The reference values have been shown in table 3.4

(a) Cholesterol ester $\xrightarrow{CHE}$ Cholesterol + Fatty acids

(b) Cholesterol + 0_2 $\xrightarrow{CO}$ Cholesterol 4en-3-one+H_20_2

(c) 2_{H202} + 4 Aminoantipyrine + Phenol $\xrightarrow{POD}$ 5 Quinoneimine dye + $4H_20$

Estimation of Triglycerides :

The estimations of serum triglycerides were done using GPO-PAP method. Triglycerides are hydrolyzed t glycerol & free fatty acids by lipase (LPL). The glycerol generated is then phosphorylated by ATP in presence of glycerol kinase (GK) to glycerol-3-phosphate which is oxidized by enzyme glycerl-3-phosphate oxidase (GPO) producing hydrogen Peroxide (H202). $_{H202}$ so formed reacts with 4-aminoantipyrine and P-cholorophenol in presence of peroxidase (POD) to produce quinonimine, an intense red chromogen, which is then measured colometrically on automated chemistry analyzers at 546 nm. The reference values are given in table 3.4

(a) Triglycerides + $_{H20}$ $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + fatty acid

(b) Glycerl + ATP $\xrightarrow{GK}$ Glyscerl-3-phosphate+ADP Mg + H_20

(c) Glycerol-3-Phosphate + 0_2 $\xrightarrow{GPO}$ H_20_2 + Dihydroxyacetone Phosphate

(d) H_20_2 + Aminoanitipyrine + DHBS $\xrightarrow{POD}$ Quinonemine + H_20

Estimation of HDL-C

Separated serum was precipitated by adding precipitating reagent (Phosphotungstic acid and dextran sulfate-magnesium chloride) after centrifugation at 3000 rpm. The supernatant was estimated for HDL-C by using chlesterol reagent (Cholesterol esterase and cholesterol oxidase) as the catalase eliminates the VLDL cholesterol, LDL cholesterol and cylomicrons. The cholesterol ester is hydrolyzed by cholesterol esterase to cholesterol and fatty acid. The cholesterl is then oxidized to cholestenone and hydrogen peroxid by chlesterol oxidase. Hydrogen peroxide in presence of peroxidase reacts with 4-aminoantipyrine and HDAOS to produce a quinine pigment. The intensity of the dye produced is directly proprtionals to the HDL cholesterl concentration when measured at 600 nm. The reference values are given in table 3.4

Estimation of VLDL

VLDL-C was calculated by diving triglycerides values by 5. The reference values are given in table 4.6.1.

VLDL = TG/5

Estimation of LDL-C

The LDL-C values were calculated using the Friedlewald's formula. The reference values are given in table 3.4

LDL-C in mg % TC - (HDL + TG/5)

TABLE 3.4 : CLASSIFICATION OF BLOOD LIPID LEVELS

	RANGE	**CLASSIFICATION**
Total Cholesterol	**< 200 mg/dl**	**Desirable**
	200-239	**Borderline**
	> 240	**High**
S.LDL-C	**<100 mg/dl**	**Optimal**
	100-129	**Near or above optimal**
	130-159	**Borderline high**
	160-189	**High**
	≥ 190 mg/dl	**Very high**
S.HDL-C	**< 40 mg/dl**	**Low**
	≥ 60 mg/dl	**High**
S.Triglycerides	**< 150g/dl**	**Normal**
	150-199	**Borderline high**
	200-499	**High**
	≥ 500	**Very high**

(ATP-III classification, 2007)

Serum Creatinine-

It is used for the determination of Creatinine in human serum/ plasma and urine (Murray R.L., 1984)

Principle-

Creatinine reacts with picric acid in an alkaline medium to form an Orange coloured complex. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinine. The reaction time and the concentration of Picric Acid and Sodium Hydroxide have been optimised to avoid interference from ketoacids.

$$\text{Creatinine + Picric Acid} \xrightarrow{\text{Alkaline medium}} \text{Orange coloured complex}$$

Refernce range (normal values)

Female 0.6-1.1 mg/dl
Male 0.9-1.3 mg/dl

Highly Sensitivity C- Reactive Protein (HsCRP)

The hsCRP ELISA is intended for the quantitative determination of C-reactive protein (CRP) in human serum. Enhanced sensitivity measurements of CRP can be useful for the detection and evaluation of infection, tissue injury, inflammatory disorders and associated diseases.

Principle:

The hsCRP ELISA 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 on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of CRP is directly proportional the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Alanine amino-transferase (ALT/GPT)

For the determination of Alanine Aminotransferase (ALT/GPT) in serum, optimised UV method (IFCC) (Thomas L., 1998).

Principle

The enzymatic reaction sequence employed in the assay of ALT is as follows:

$$\text{L-Alanine + a-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-Glutamate}$$

$$\text{LDH Pyruvate + NADH + H+} \xrightarrow[\text{LDH}]{} \text{Lactate + NAD+ + } H_2O$$

The Pyruvate formed in the first reaction is reduced to lactate in the presence of lactate dehydrogenase and NADH. The activity of ALT is determined by measuring the rate of oxidation of NADH at 340nm. Endogenous sample pyruvate is converted to lactate by LDH during the lag phase prior to measurement.

Reference values (normal values)

SGPT- Up to 26 IU/L (30°C)
SGOT-Up to 38 IU/L (37°C)

Total Protein

Principle

The Peptide bonds of Proteins react with Cupric ions in alkaline solution to form a colored chelate, the absorbance of which is measured at 578 nm. The Biuret Reagent contains Sodium-potassium Tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final color is proportional to the concentration of Total protein in the sample (Koller A., 1984).

$$\text{Protein + Cu"} \xrightarrow{\text{Alkaline pH}} \text{Cu-Protein Complex}$$

Calculation

$$\text{Total Protein Concentration (g/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 6.5$$

Globulins = Total Protein - Albumin

Conversion factor

Total Protein Concentration in g/L = Total Protein Concentration in g/dL x 10

TABLE: REFERENCE RANGE FOR TOTAL PROTEIN

Age group	Serum Total Protein Concentration in g/dl
Cord	4.8-8.0
Premature	3.6-6.0
New born	4.6-7.0
1 week	4.4-7.6
7 Months to 1 year	5.1-7.3
1 to 2 years	5.6-7.5
3 years	6.0-8
Adults	6.4-7.8
60 years	< 0.2

Albumin

Principle

At P^H 3.68, Albumin acts as action and binds to the anionic dye Bromocresol Green (BCG) , forming a green coloured complex. The absorbance of final colour is measured at 630 nm. The colour intensity of the complex is proportional to Albumin concentration in the Sample (Gendler., 1984).

$$\text{Albumin} + \text{BCG} \xrightarrow{P^H\ 3.68} \text{Green Colored Complex}$$

TABLE : REFERENCE RANGE FOR SERUM ALBUMIN

Age group	Serum Albumin Concentration in g/dL
0-4 days	2.8-4.4
4 days to 14 years	3.8-5.4
Adults	3.5-5.2
> 60 years	3.2-4.6

ANNEXURE - 7

GLIMPSES OF INTERACTION WITH THE ENROLLED SUBJECTS