APPENDIX – I
A QUESTIONNAIRE TO ELICIT THE INFORMATION REGARDING THE FORMULATION OF RISK INDEX AND DIET COUNSELLING FOR WOMEN WITH BREAST CANCER

Name of the Investigator : Date:
Name of the Interviewee : 

SOCIO-ECONOMIC BACKGROUND:
1. Name :
2. Address :
3. Education :
   Primary □ Secondary □ Higher Secondary □
   Graduate □ Illiterate □ Others □
4. Occupation : Home Maker □ Working □
5. Height ........cm Weight.........kg 
6. Age...........years 
7. Residence : Urban □ Rural □
8. Marital Status : Married □ Single □ Widow □
9. Type of family : Joint □ Nuclear □
10. Size of family : ........Adults...........Children 
11. Total monthly income of the family:

PERSONAL DATA :

A) MENSTRUAL HISTORY:-
1. Age at menarche
   < 10 yrs. □ 10-12 yrs. □ 12-14 yrs. □ > 14 yrs. □
2. Tick the pre-menstrual syndrome that you suffer during every period.
   Abdominal pain □ Muscular Cramps □ Tiredness □
   Vomiting □ Irritability □ Headache □ Others specify
3. Is your menstrual cycle: Regular ☐ Irregular ☐
   If yes, Normal ☐ Normal ☐
   Heavy ☐ Heavy ☐
   Scanty ☐ Scanty ☐

4. If irregular, Tick the duration between the cycle
   once in 15 days ☐ once in 45 days ☐
   once in 2 months ☐ others specify ☐

5. Treatment taken for irregular menstrual cycle
   i) Allopathy ☐ Homeopathy ☐ Ayurvedic ☐ Dietary ☐
      Combinations specify.
   ii) Are you continuing the support? Yes/No. If yes, mention the drug.

6. Duration of the menstrual cycle
   3 days ☐ 4 days ☐ 5 days ☐ more than 5 days ☐

7. Number of days of menstrual cycle/month
   < 28 days ☐ 28 days ☐ >28 days ☐ others specify ☐

8(i) Did you have the habit of preponing / post poning menstrual cycle? Yes / No.
    If yes, reason.

8(ii) Mention the type of medical support you take for this.
      Allopathy ☐ Homeopathy ☐ Ayurvedic ☐
      Dietary ☐ Combinations, specify ☐

B) FAMILY LIFE DETAILS:-
1. Mention your age at marriage………yrs
2. Mention the age at first child birth………yrs.
3. Have you used contraceptives? Yes / No.
   If yes,
   Before first child birth ☐ After first child birth ☐ Continuing the use ☐

4. Mention the type of contraceptive you use
   Oral pills ☐ Copper ☐ Preventol ☐ any other drugs ☐
   Specify, the drug duration dose taken.

5. Mention the number of
   Miscarriage ☐ Still birth ☐ Abortion ☐ None ☐
C) **BREAST FEEDING PRACTICES:**

1. Mention the mode of delivery.
   
   1st child Normal / Cesarean
   2nd Child Normal / Cesarean

2. Was your child fed with colostrum
   
   Yes ☐ No ☐

3. When was the breast feeding initiated
   
   within ½ hour ☐ After one day ☐ 1 hour ☐ Later ☐

4. How long was your baby fed with breast milk?

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<tr>
<th>Duration of feed (months)</th>
<th>Order of child</th>
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<td>1st</td>
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<td>3 – 6</td>
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<td>Not feed</td>
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5. Tick the reason for not breast feeding.
   
   Small size breast ☐ Infections / Wouxe in breast ☐
   Feels insufficient milk secretion ☐ Not healthy ☐
   Family circumstances ☐ Working women ☐
   Myths ☐ mention ..........

6. Mention the practice adopted to initiate /increase / stop the breast milk secretion.
   
   i) Drug ☐ specify
   ii) Diet ☐ specify
   iii) Any others ☐ specify

D) **POST MENOPAUSAL WOMEN:**

1. Age at menopause :

2. Pick the post menopausal symptoms

   Irregular periods ☐ Dizziness ☐
   Hot flush ☐ Irritability ☐
   Depression ☐ All the above ☐
   None of the above ☐ Others, specify ☐
3. Treatment taken from the onset of symptoms
   - Allopathy
   - Homeopathy
   - Ayurvedic
   - Siddha
   - Hormonal therapy
   - Diet
   - No treatment
   - Combination, specify

4. Complication after menopause:
   - Obese
   - Hypertension
   - DM
   - Osteoporosis
   - Cancer
   - Other specify

III. LIFE STYLE PATTERN:

1. Do you have the habit of tobacco chewing Yes / No?
   - If yes, mention the type of tobacco
     - Pann
     - Gutka
     - Raw tobacco with betel

2. How long do you sleep / day?
   - < 5 hrs
   - 5-7 hrs
   - >7 hrs

3. Mention the hours you spend for the daily activities?

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<thead>
<tr>
<th>Sl.No.</th>
<th>Activities</th>
<th>Total hours spent</th>
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<tbody>
<tr>
<td>1.</td>
<td>Washing clothes</td>
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<td>Washing dishes</td>
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<td>3.</td>
<td>Cooking</td>
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<td>4.</td>
<td>Gardening</td>
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<td>5.</td>
<td>Watching TV</td>
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<td>Reading</td>
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<td>8.</td>
<td>Exercise</td>
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<td>9.</td>
<td>Outing</td>
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<td>10.</td>
<td>Others, specify</td>
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</tbody>
</table>

4. Do you exercise regularly, Yes / No.
   - If yes, mention the type and duration.
     - Type of Exercise
     - Duration in Min. < 20 20-40 40-60 > 60
       - Walking
       - Aerobics
       - Gym
       - Yoga
       - Meditation

IV. ENVIRONMENTAL EXPOSURE

1. Do you come across usage of any chemicals in daily life ? Yes / No.
2. If yes, where, □ At Home □ at work spot □ both places

3. Frequency of using

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<tr>
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<th>Daily</th>
<th>Weekly</th>
<th>Fortnightly</th>
<th>Occasionally</th>
<th>Rarely</th>
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<td>At home</td>
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<td>At work</td>
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4. Mention the type of chemicals? _________________

5. How long you are using mosquito repellents? _____ years ___ months / day.

V. MEDICAL HISTORY

1. Detection of condition through
   - Routine checkup □
   - Screening Tests □
   - Self – Examination □
   - Others, Specify □

2. When was it detected? ______ yrs / ______ Months.

3. Did any of your family members suffered from this condition? Yes / No.
   - If yes (Tick) Mother □ Father □
   - Maternal Aunt □ Paternal Aunt □
   - Maternal Uncle □ Paternal Uncle □
   - Grand Mother □ Grand Father □

4. '√' the symptoms you have / had.
   - Change in normal size of breast.
   - Change in position of nipple.
   - De pigmentation of breast / nipple.
   - Abnormal Secretions from breast
   - Lumps □ pain □ infections / wound.

5. Type of treatment you are undergoing.
   - □ Allopathy □ Homeopathy □ Chemotherapy
   - □ Ayurvedic □ Radiation
   - □ Hormonal Therapy. □ Combinations, Specify

6. Are you continuing the treatment? Yes / No.

7. Rank the Carcinogen according to your use?
   - □ Coffee □ Tobacco
   - □ Broiled Meat / Chicken □ Improper stored grains
VI. DIETARY PRACTICES AND PATTERNS:-

1. Are you a Vegetarian / Non – Vegetarian / Lacto – Veg / Ovo – Veg / Fish eaters.

2. Do you use black colour plastic covers for storage? Yes / No.
   If yes, mention the type of food stored?

3. How often you purchase

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<thead>
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<td>5. Pork</td>
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<td>6. Beef</td>
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</table>

4. Which method do you adopt for cooking Non-vegetarian foods?
   a) Boiling ☐
   b) Frying ☐
   c) Broiling ☐
   d) Steaming ☐
   e) Deep fat frying ☐
   f) Grilling ☐
   g) Roasting ☐
   h) Microwave cooking ☐
   i) Combination of steaming & frying ☐
   j) Any other Combination specify ☐

5. What type of oil you use for cooking.
   a) Coconut oil ☐
   b) Gingelly oil ☐
   c) Sunflower oil ☐
   d) Groundnut oil ☐
   e) Corn oil ☐
   f) Combinations Specify.

6. What do you do with the left over cooking oil.
7. Have you have the habit of reusing the cooked oil?  
   Yes / No, If yes, after how long _________________.

8. Mention the type of beverage you consume  
   ☐ Tea ☐ Coffee ☐ Milk  
   ☐ Other health drinks ☐ None ☐ All

9. How many times per day you consume Coffee / Tea.  
   ☐ Twice ☐ More ☐ Thrice

10. Do you use preservatives in your cooking . Yes / No.  
    If yes, mention the item  
    ☐ MSG ☐ Vinegar ☐ Sodium Benzoate  
    ☐ KMS ☐ Coloring agents.

11. Mention the most preferred soft drink  
    1.  
    2.  
    3.  
    4.  
    5.

12. Mention the preferred items when  
    At Hotel At Fast Food Center
    | Name of the Item | Frequency | Name of the Item | Frequency |
    |------------------|-----------|------------------|-----------|
    | 1.               |           | 1.               |           |
    | 2.               |           | 2.               |           |
    | 3.               |           | 3.               |           |
    | 4.               |           | 4.               |           |

Three day Dietary Recall.  

1 Day

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## APPENDIX – II

### ASSESSMENT OF CLINICAL SIGNS

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<th>Part</th>
<th>Description</th>
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<th>Scanty</th>
<th>Puffy</th>
<th>Sickly look</th>
<th>Red coloured</th>
<th>Down slanted</th>
<th>Angular stomatitis</th>
<th>Pale and flabby</th>
<th>Glossitis</th>
<th>Dry and scaly</th>
<th>Hyper pigmentation</th>
<th>Rashes</th>
<th>Mottled enamel</th>
<th>Caries</th>
<th>Wide spaced</th>
<th>Enlarged abdomen</th>
<th>Spoon shaped</th>
<th>Thyroid enlargement</th>
<th>No thyroid</th>
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<td>Gland enlargement</td>
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<td>Knock knees</td>
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APPENDIX – III

ASSESSMENT OF NUTRITIONAL STATUS USING SCORED
PG-SGA TOOL

Scored Patient-Generated Subjective
Global Assessment (PG-SGA)

History: (Boxes 1-4 are designed to be completed by the patient.)

1. Weight: (See Worksheet 1)

   In summary of my current and recent weight:
   - I currently weigh about ________ kg
   - I am about ________ cm tall
   - One month ago I weighed about ________ kg
   - Six months ago I weighed about ________ kg
   - During the past two weeks my weight has:
     - decreased
     - not changed
     - increased

2. Food Intake: As compared to my normal intake, I would rate my food intake during the past month as:
   - unchanged
   - more than usual
   - less than usual
   - I am now taking:
     - normal food
     - little solid food
     - only liquids
     - only parenteral or enteral nutrition
     - very little of anything
     - only tube feedings or only nutrition by vein

3. Symptoms: I have had the following problems that have kept me from eating enough during the past two weeks (check all that apply):
   - no problems eating
   - no appetite, just didn't feel like eating
   - nausea
   - constipation
   - diarrhea
   - mouth sores
   - dry mouth
   - things taste funny or have no taste
   - smell bothers me
   - problems swallowing
   - feel full quickly
   - pain where?
   - other

4. Activities and Functions: Over the past month, I would generally rate my activity as:
   - normal with no limitations
   - not my normal self, but able to be up and about with fairly normal activities
   - not feeling up to most things, but in bed or chair less than half the day
   - able to do little activity and spend most of the day in bed or chair
   - pretty much bedridden, rarely out of bed

   Additive Score of the Boxes 1-4: A

The remainder of this form will be completed by your doctor, nurse, or therapist. Thank you.

5. Disease and its relation to nutritional requirements: (See Worksheet 2)

   All relevant diagnoses (specify)

   Primary disease stage (circle if known or appropriate) I II III IV Other

   Numerical score from Worksheet 2: B

6. Metabolic Demand: (See Worksheet 2)

   Numerical score from Worksheet 3: C

7. Physical: (See Worksheet 4)

   Global Assessment: (See Worksheet 5)

   Total PG-SGA score: Total numerical score of A+B+C+D above

   (See triage recommendations below)

   Nutritional Triage Recommendations: Additive score is used to define specific nutritional interventions including patient & family education, symptom management including pharmacologic intervention, and appropriate nutrition intervention (food, nutritional supplements, enteral, or parenteral routes). First line nutrition intervention includes optimal symptom management.

   - No intervention required at this time. Re-assessment on routine and regular basis during treatment.
   - Requires intervention by dietitian, nurse, or other clinician with pharmacologic intervention as indicated by symptoms survey (Box 3) and laboratory values as appropriate.
   - Requires intervention by dietitian, in conjunction with nurse or physician as indicated by symptoms survey (Box 3).

   Indicates a critical need for improved symptom management and/or nutritional intervention options.
Worksheets for PG-SGA Scoring

Worksheet 1 - Scoring Weight (Wt) Loss

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<thead>
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<th>Wt loss in 1 month</th>
<th>Points</th>
<th>Wt loss in 6 months</th>
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<td>4</td>
<td>20% or greater</td>
</tr>
<tr>
<td>5-9%</td>
<td>3</td>
<td>10-19%</td>
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<tr>
<td>3-4%</td>
<td>2</td>
<td>6-9%</td>
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<tr>
<td>2-2%</td>
<td>1</td>
<td>2-5%</td>
</tr>
<tr>
<td>0-1%</td>
<td>0</td>
<td>0-2%</td>
</tr>
</tbody>
</table>

Score for Worksheet 1 = [Box 1]

Record in Box C

Worksheet 2 - Scoring Criteria for Condition

Score is derived by adding 1 point for each of the conditions listed below that pertain to the patient:

Category
- Cancer
- AIDS
- Pulmonary or cardiac cachexia
- Presence of decubitus, open wound, or fistula
- Presence of trauma
- Age greater than 65 years

Score for Worksheet 2 = [Box D]

Record in Box B

Worksheet 3 - Scoring Metabolic Stress

Score for metabolic stress is determined by a number of variables known to increase protein & calorie needs. The score is additive so that a patient who has a fever of >102 degrees (3 points) and is 16 mg of prednisone clinically (2 points) would have an additive score for this section of 5 points.

<table>
<thead>
<tr>
<th>Stress</th>
<th>none (0)</th>
<th>low (1)</th>
<th>moderate (2)</th>
<th>high (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>no fever</td>
<td>&lt;100 and &lt;101</td>
<td>≥101 and &lt;102</td>
<td>≥102</td>
</tr>
<tr>
<td>Fever duration</td>
<td>no fever</td>
<td>&lt;72 hrs</td>
<td>moderate dose</td>
<td>&gt;72 hrs</td>
</tr>
<tr>
<td>Steroids</td>
<td>no steroids</td>
<td>low dose</td>
<td>dose (≤30mg prednisone equivalents/day)</td>
<td>high dose steroids</td>
</tr>
</tbody>
</table>

Score for Worksheet 3 = [Box C]

Record in Box C

Worksheet 4 - Physical Examination

Physical exam includes a subjective evaluation of 3 aspects of body composition: fat, muscle, & fluid status. Since this is subjective, each aspect of the exam is rated for degree of deficit. Muscle deficit impacts point score more than fat deficit. Definitions of categories: 0 = no deficit, 1 = mild deficit, 2 = moderate deficit, 3 = severe deficit. Ratings of deficit in these categories are not additive but are used to clinically assess the degree of deficit (or presence of excess fluid).

Fat Status
- Subcutaneous fat pad
- Strips skin fold
- Fat overlying lower ribs
- Global fat deficit rating

Muscle Status
- Triceps (temporal muscle)
- Gluteal muscles (iliopsoas & gluteus medius)
- Thighs (sartorius, adductors)
- Abdominal muscles
- Hand grip strength
- Calf girth (gastrocnemius)
- Global muscle status rating

Point score for the physical exam is determined by the overall subjective rating of total body deficit.

No deficit score = 0 points
Mild deficit score = 1 point
Moderate deficit score = 2 points
Severe deficit score = 3 points

Score for Worksheet 4 = [Box D]

Record in Box D

Worksheet 5 - PG-SGA Global Assessment Categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Stage A</th>
<th>Stage B</th>
<th>Stage C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>No wt loss OR Recent non-fluid wt gain</td>
<td>&lt;5% wt loss within 1 month (or 10% in 6 months) OR No wt stabilization or wt gain (i.e., continued wt loss)</td>
<td>&gt;5% wt loss in 1 month (or &gt;10% in 6 months) OR No wt stabilization or wt gain (i.e., continued wt loss)</td>
</tr>
<tr>
<td>Nutrient Intake</td>
<td>No deficit OR Significant recent improvement</td>
<td>Moderate functional deficit OR Recent deterioration</td>
<td>Severe deficit in intake</td>
</tr>
<tr>
<td>Nutrition Impact Symptoms</td>
<td>None OR Significant recent improvement allowing adequate intake</td>
<td>Evidence of mild to moderate loss of SQ fat, loss of muscular mass, loss of muscle tone or palpation</td>
<td>Evidence of severe malnutrition (e.g., severe loss of SQ tissue, possible edema)</td>
</tr>
</tbody>
</table>

Global PG-SGA rating (A, B, or C) = [Box E]
APPENDIX – IV

APPROVAL OF THE HUMAN ETHICS COMMITTEE

PROPOSAL NUMBER : 08/96/NCT/Jyothi/Nutritional Deficiency

PROJECT TITLE:
Identification of Nutritional Deficiency and the Effect of Antioxidants in selected cancer patients

NAME OF THE INVESTIGATOR : Ms V Jyothi

NAME OF THE CO-INVESTIGATOR : Ms S Alamelu Mangai

NAME OF THE GUIDE : Dr V Sharadha Ramadas

REVIEW TYPE : Exempt

DATE OF THE MEETING : NA

DECISION : Approved in the present form

APPROVAL DATE : 29-12-08

VALIDITY OF THE APPROVAL : One year

CONTINUING PANEL REVIEW : Not Needed

Dr S Bhuvaneshwari
Secretary
Institutional Human Ethics Committee
**APPENDIX – VI**

**CHECK LIST TO ASSESS THE KNOWLEDGE OF SUBJECTS ON VARIOUS ASPECTS OF CANCERS**

1. Where you aware of breast cancer as a disease? Yes / No.

2. Mention the early warning signs that you are aware.

- Breast lump
- Painless lump
- Pain in breast
- Breast abscess
- Nipple Discharge
- Orange peel like appearance
- Change in size of breast
- Change in position of nipple
- Depigmentation of breast / nipple
- Swelling in axilla

3. Identify the risk factors of breast cancer by ‘√’ against the boxes.

- Early menarche age
- Irregularity of menstrual cycle
- Late marriage
- Use of contraceptives
- Late age at first child birth
- Hystectomy
- Nulliparity
- Not breast fed
- Late menopause
- Obesity
- Raw tobacco
- Passive smoking
- Exposure to chemicals
- Meat consumption
- High fat diet
- Use of reheated oil
- Use of colours / preservatives in diet
- Not aware of any risk factors

4. ‘√’ the preventive measure

- Regular check up by a doctor
- Healthy diet
- Physical exercise
- Maintenance of Optimum BMI
- Others, Mention.

5. ‘√’ against the sources from which you had heard about breast cancer.

- Television
- Radio FM
- Print Material
- Neighbours, friends and relatives
- Health care professionals

6. Are you aware of foods to be included: Yes / No.

7. Are you aware of foods to be excluded during the treatments. Yes / No.

8. Are you aware of significance of antioxidant foods. Yes / No.

9. Do you know the importance of right selection of foods. Yes / No.

10. Do you think that vegetarian diet is good for health. Yes / No.
APPENDIX – VII
SOFTWARE C.D.
APPENDIX – VIII

PROCEDURES

DETERMINATION OF CAROTENES IN SERUM USING THE CARR-PRICE REACTION
(Kimble, 1938)

Proteins are precipitated with ethanol and carotenes extracted into light petroleum. After reading the intensity of the yellow, colour due to the carotenes the light petroleum is evaporated off and the residue dissolved in chloroform. Carr-Price reagent is added and the blue colour produced red. Since carotenes also give some colour, a correction for this made in order to obtain the retinol present.

Reagents

1. Absolute ethanol
2. Light petroleum (boiling point 40-60 days)

Technique

Pipette 3 ml serum into a stoppered centrifuge tube add 3 ml absolute ethanol, slowly drop by drop with shaking, in order to obtain a finely divided precipitate of protein. Add 6 ml light petroleum and shake vigorously for 10 minutes, then centrifuge at a low speed for about one minute. Pippette off as much as possible of light petroleum layer, taking care not to remove any of the water layer with it.

Place the light petroleum extract in the calorimeter cuvette and read at 440 nm or with violet filter using petroleum as blank.

Calculation

\[
\text{Beta carotene} = \frac{T}{134} \times 1 \times 537
\]

Beta carotene was calculated using its molar extinction co-efficient at 435 (134000) and expressed in terms of mg/l by multiplying with its molecular weight.

ESTIMATION OF ASCORBIC ACID
(Omaye, 1971)

Aim

To estimate the amount of ascorbic acid in the given sample unknown solution.

Principle

Ascorbic acid is oxidized to dehydro ascorbic acid by bromine water. The bromine is removed by a aeration. The dehydro ascorbic acid is heated with thiourea and then coupled with 2, 4 – dinitrophenyl hydrazine and finally heated with 85 per
cent sulphuric acid to produce a red colour which is measured colorimetrically at 540 nm.

**Reagents**

1) 2 per cent solution of 2, 4, dinitrophenyl hydrazine in 9 N sulphuric acid.
2) 10 per cent thiourea solution
3) 85 per cent sulphuric acid
4) 4 per cent oxalic acid
5) Stock standard ascorbic acid : 100 mg of ascorbate was weighed accurately and made upto the mark with four per cent oxalic acid.
6) Working standard ascorbic acid : 10 ml of the stock standard was pipetted out into 100 ml standard flask and made upto the mark with four per cent oxalic acid. From this 10 ml was taken and transferred to a clean dry conical flask and added a few drops of bromine water till the solution become yellow.

The excess bromine was removed by aeration and the solution was made upto 50 ml with four per cent oxalic acid, 1 ml of this solution contained 20 μg of oxalic acid.

**Procedure**

Known volumes of the standard solution (0.5 – 2.5 ml) corresponding to the concentrations of 10, 20, 30, 40 and 50 μg of ascorbic acid were taken in a series of test tubes.

To all the test tubes added a drop of thiourea solution and 1 ml of 2, 4, -dihydrophenyl hydrazine reagent. Made up the volume each tube to 6.0 ml with 4 per cent oxalic acid. Incubated for 3 hours at 37°C. removed the tubes and cooled them in ice. Added 4 ml of 85 per cent sulphuric acid to each of the tubes and the red colour developed was read against a reagent blank at 540 nm. From the standard graph curve constructed on a scientific calculator using the linear regression made calculated the amount of ascorbic acid in 100 ml of the unknown solution.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solution</th>
<th>Volume of 2,4-dinitrophenyl hydrazine (ml)</th>
<th>Volume of oxalic acid (ml)</th>
<th>Volume of 85% H₂SO₄ (ml)</th>
<th>Optimal density at 540 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blank Standard</td>
<td>-</td>
<td>1.0</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>2.</td>
<td>Blank Standard</td>
<td>0.5</td>
<td>1.0</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>Blank</td>
<td>1.0</td>
<td>1.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma</td>
<td>1.5</td>
<td>1.0</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma</td>
<td>1.5</td>
<td>1.0</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma</td>
<td>1.5</td>
<td>1.0</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma</td>
<td>1.5</td>
<td>1.0</td>
<td>3.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>
**Calculation**

Klett reading .......... corresponds to ..............μg of ascorbic acid

1.5 ml of plasma contain ...............μg of ascorbic acid

∴ 1000 ml of plasma contain = \[
\frac{\text{.......... x 1000}}{1.5 \times 1000}
\]

= ............... mg of ascorbic acid

**DETERMINATION OF SERUM TOCOPHEROL**

*(Baker and Frank, 1968)*

**Reagents :**

1. Absolute ethanol, aldehyde free
2. Xylene
3. α-α′ Dipyridyl, 1.20 g/l in n-propanol
4. Ferric chloride solution, 1.20 g FeCl₃, 6 H₂O/l ethanol. Keep in a brown bottle.
5. Standard solution of D-tocopherol, 10 mg/l in ethanol.

**Technique**

Into three stoppered centrifuge tubes measure 1.5 ml serum, 1.5 ml standard and 1.5 ml H₂O (blank) respectively. To test and blank and 1.5 ml ethanol and to standard 1.5 ml H₂O. Then add 1.5 ml xylene to all the tubes, stopper, mix well and centrifuge. Transfer 1 ml of the xylene layers into other stoppered tubes taking care not to include any ethanol or protein. Add 1 ml α-α′ dipyridyl reagent to each tube, stopper and mix. Pipette 1.5 ml of the mixture into colorimeter cuvettes and read the extinction of test and standard blank at 460 nm. Then in turn beginning with the blank add 0.33 ml of ferric chloride solution, mix and after exactly 1.5 minutes, read test and standard against the blank at 520 nm.

**Calculation**

Serum tocopherols (mg/l) \[
\frac{\text{reading of unknown at 520 nm} - \text{reading at 460 nm} \times 0.29}{\text{reading of standard at 520 nm}} \times 10
\]

Since the standard contain 10 mg/l.

**ESTIMATION OF GLUTATHIONE PEROXIDASE**

*Colorimetric method*

*Rotruck et al. (1984)*

**Principle**

Glutathione peroxidase activity is determined by assaying the residual content of reduced glutathione which produces an yellow colored compound with 5,5 dithiobis 2 nitrobenzoic acid that absorbs at 412 nm.
Reagents

1. Sodium phosphate buffer: 0.4 M, pH 7.0
2. Sodium azide: 10 mM
3. Reduced glutathione: 4 mM
4. Hydrogen peroxide: 2.5 mM
5. Trichloro acetic acid: 10 % solution
6. Phosphate solution: 0.3 mM disodium hydrogen phosphate
7. DTNB reagent: 40 mg of 5,5 dithiobis (2-nitrobenzoic acid)/100 ml of 1 % sodium nitrate.
8. Reduced glutathione standard: 20 mg % solution

Procedure

0.2 ml buffer, 0.05 ml sodium azide, 0.1 ml reduced glutathione, required amount of enzyme, 0.05 ml hydrogen peroxide and water were taken to a final incubation volume of 2 ml. The tubes were incubated at 30°C for 10 minutes. The reaction was terminated by the addition of 0.025 ml TCA. To determine the residual GSH content, the supernatant was removed by centrifugation and added to 1.5 ml of disodium hydrogen phosphate and 0.5 ml of DTNB reagent. The colour was read at 412 nm. A blank was prepared with only sodium dihydrogen phosphate and 1 ml of DTNB reagent. Suitable aliquots of the standard were taken and treated in the same manner.

ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY

Colorimetric method
Misra and Fridovich (1972)

Principle

Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of Nitro Blue Tetrazolium (NBT) reduction, the extent of which can be assayed spectrophotometrically at 430 nm.

Reagents

1. 50 mM potassium phosphate buffer, pH 7.8
2. 45 μM methionine
3. 5.3 mM riboflavin
4. 84 μM NBT
5. 20 μM potassium cyanide

Procedure

The incubation medium contained in a final volume of 3 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 μM methionine, 5.3 mM riboflavin, 84 μM NBT and 20 μM potassium cyanide. The amount of serum added to this medium was kept below 1 unit of enzyme to ensure sufficient accuracy.
The tubes were placed in an aluminium foil lined box maintained at 25°C. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT.

ESTIMATION OF HAEMOGLOBIN LEVELS BY CYANMETHAEMOGLOBIN METHOD USING FILTER PAPER TECHNIQUE

Principle

Ferrous ions (Fe++) ions of haemoglobin are oxidized to ferric (Fe+++ ) ions by potassium ferricyamide to form methaemoglobin. This methaemoglobin reacts with cyanide (CN-) ions of potassium cyanide to form cyanmethaemoglobin.

Procedure for Estimation of Hb

A. Preparation of Drabkin’s Solution

Adding 2.5 ml of Drabkin’s concentrate to 47.5 ml of distilled H2O gives 50 ml of Drabkin’s solution. This amount is enough for 10 samples to be analysed.

B. Preparation of the Sample

1. Pipette out exactly 5 ml of the diluted Drabkin’s solution.
2. Cut carefully the portion of filter paper with the blood spot and transfer it to a precoded test tube having 5 ml of Drabkin’s solution.
3. Allow the filter paper with the blood spot to soak overnight in Drabkin’s reagent for complete extraction of the blood. Now the filter paper appears white.

C. Estimation of Haemoglobin

1. Set the reading to ‘0’ by rotating the ‘set zero’ adjustment knob.
2. Take Drabkin’s reagent in a cuvette (upto 3/4th) and place it in the well. Adjust the reading to 100 by rotating the set 100 adjustment knob.
3. Do not adjust the settling till all readings are finished.
4. Shift the switch to “OD” (Optical Density) mode before taking readings of standard and samples.
5. Take readings on standard solution provided at the beginning and the end.
6. Mix the sample in the test tube well, before transferring into glass cuvette (upto 3/4th).
7. Transfer the contents of the test tube with the sample into the cuvette and take the readings.
8. Wipe the sides of the cuvette before keeping in the instrument.
9. Enter the sample OD in the register against the correct identification number.

D. Calculation of Haemoglobin Concentration

The following formula is used.

\[
\text{Haemoglobin (g/dl)} = \frac{\text{OD of sample} \times \text{concentration} \times \text{standard} \times 251}{\text{OD of standard} \times 1000}
\]

where concentration of haemoglobin standard = 60 mg/dl dilute factor = 251.