

# *Methods & Materials*

## **Methods & Materials**

Malnutrition is the world's most grave health problem and the single biggest contributor to child mortality. Under nutrition is not just a state, but a process whose consequences often extend not only in later life, but also into future generations. Deficiencies of key vitamins and minerals continue to be pervasive and they overlap considerably with problem of general under nutrition. The prevalence of underweight children in India is among the highest in the world. This period is characterized by an exceptionally rapid rate of growth. School provides the most effective and efficient way to reach large portion of the population. MDM (Mid Day Meal) is currently going on all over India and in Gujarat. We need to relook at the prevalence of malnutrition and IDA (Iron Deficiency Anemia) and come out with cost effective remedial techniques to tackle it. Thus the present study was carried out in the rural government schools of Vadodara to study the growth dynamics of rural school children over a period of 3 years and to see the impact of weekly IFA tablet and deworming tablet on the growth, hemoglobin and physical work capacity of children.

### **MEDICAL ETHICS AND APPROVALS**

The study was approved by the Institutional Medical Ethics Committee (Approval no. FCSc./FND/ME/45 dated 30/11/2009).

The following approvals were obtained for the study:

1. Permission from District Education Officer, Vadodara.
2. Permission from Principals of schools.
3. Individual consent from parents of school children for drawing blood.

## PHASES OF STUDY

The study was divided into three phases

**Phase 1:** Formative Research on nutritional status of school going children of rural Vadodara

**Phase 2:** To study the growth dynamics of children by longitudinal data (three years).

**Phase 3: A** Impact evaluation of Weekly IFA tablet and deworming tablet on the growth, hemoglobin and physical work capacity of children.

**Phase 3: B** The long term impact of the intervention for the period of 6 months.

## SAMPLE SIZE ESTIMATION

For the number of children to be included in a research study, the sample size of the study is an important consideration in designing the research. The simple formula for determining the sample size is as follows:

$$N=16p (100-p)/w^2$$

Where,

P = estimated prevalence based on earlier study or pilot trial

W= Width of confidence interval

e.g. if the interval is 95 % the width will be 10 (±5)

Now for the following study, considering the prevalence of malnutrition to be 60 % the sample size was estimated as follows:

$$\begin{aligned} N &= 16 \times 60 (100-60) / 10^2 \\ &= 384 \end{aligned}$$

Thus the sample size for the study was taken keeping the prevalence rate of malnutrition in Vadodara in children (G.W. Lasker & C.G.N. Mascie Taylor, 1993).

## **PHASE 1: NUTRITIONAL ASSESSMENT OF THE RURAL CHILDREN**

The major objective of doing the cross sectional study was to assess the magnitude of malnutrition and anemia problem among adolescent school children and to arrive at the determinants of it.

### **SAMPLE SELECTION**

The present study was conducted in the rural petrochemical area of Vadodara district, Gujarat. The petrochemical area was divided into six identical zones. All the schools which gave permission to carry out the study were taken. One representative school from each zone was randomly selected. All the children from 1<sup>st</sup> to 7<sup>th</sup> standard of the school were enrolled for the study. The total number of registered children was 3170 out of which data could be collected on 2282 children. Exclusion criteria included the children who could not be contacted in 3 consecutive visits. There was almost 28 % of absenteeism in rural schools. The rate of absenteeism was high in the rural area as parents were not that proactive in sending their children to school. For some children the school was far off so they avoided coming to the school. In the rural area the problem of migration for livelihood is also there. The experimental plan for phase 1 is given in Figure 3.1

Anthropometric measurements were done in all the schools. All the children from 1<sup>st</sup> to 7<sup>th</sup> standard were assessed. The questionnaire on dietary pattern was to be administered in school itself. Children of 1<sup>st</sup> to 3<sup>rd</sup> standard were too small to accurately report their dietary pattern. So students from 4<sup>th</sup> to 7<sup>th</sup> standard were assessed. The main aim of clinical sign and symptom was to see the co-relation between invasive and non invasive method. So clinical sign and symptoms assessment was done in 3 schools in which intervention was to be done. All the children from 1<sup>st</sup> to 7<sup>th</sup> standard were assessed. For hemoglobin estimation,

students from 4<sup>th</sup> to 7<sup>th</sup> standard were enrolled. Younger children of 1<sup>st</sup> to 3<sup>rd</sup> standard were not at all supportive for invasive technique so were not involved.

The following data was collected on all the children:

- A) Socio-economic data (Annexure 1)
- B) Anthropometric data (Height, Weight, Waist & Hip circumference)
- C) Clinical signs and symptoms (Annexure 2 )
- D) Three day dietary pattern (Annexure 3)
- E) Biochemical estimation of Hemoglobin

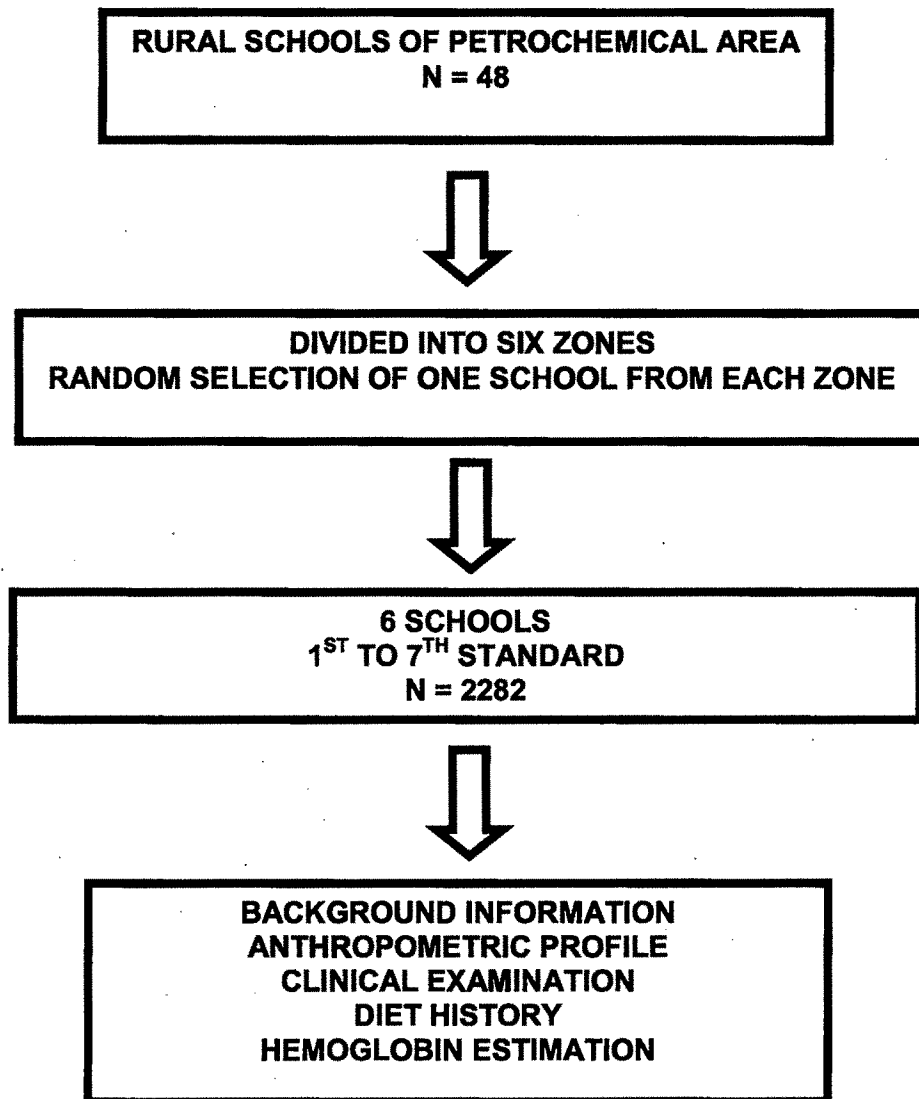
The Table below gives an overview of sample distribution

**Table 3.1: Overview of sample distribution**

Parameters	No. of Schools	Standard	Sample Size
Anthropometric measurements	6	1 <sup>st</sup> to 7 <sup>th</sup>	2282
3 Day Dietary pattern	4	4 <sup>th</sup> to 7 <sup>th</sup>	906
Clinical signs and symptoms	3	1 <sup>st</sup> to 7 <sup>th</sup>	960
Hemoglobin	4	4 <sup>th</sup> to 7 <sup>th</sup>	865

**FIGURE 3.1: EXPERIMENTAL PLAN**

**PHASE I: FORMATIVE RESEARCH ON NUTRITIONAL STATUS OF  
SCHOOL GOING CHILDREN**



**DATA COLLECTION**

The tools used to elicit the data collection are given below

**Table 3.2: Tools used for data collection**

Indicator	Procedure
Socioeconomic status	Structured interview
Weight	Digital Bathroom Scale
Height, waist, Hip	Fiber glass tape
Dietary Pattern	Structured interview
Hemoglobin	Cyan met hemoglobin method
Clinical signs and symptoms	Structured interview

**METHOD**

**1) Socio- economic status**

Information on the socio- economic profile was collected using a pre-tested structured questionnaire (Appendix 1). Information regarding age, sex, religion, family members, parent’s education and occupation, per capita income etc. was collected. Information on date of birth of children was verified from the school records. Some socio economic information like income, occupation of parents were also available in school records.

**2) Anthropometry**

**A) Weight**

Weight measurement was done for all the children using a calibrated digital weighing scale. It is portable and can be conveniently used in the field. The subject was asked to stand erect on the scale without touching anything, with no

heavy clothing and footwear and looking straight ahead. The instrument was calibrated daily using 5kg sand bag.

### **B) Height**

Height measurements of all the subjects were taken using a flexible, non-stretchable fiber glass tape. The tape was fixed vertically on a smooth wall of the school perpendicular to the ground, ensuring that the floor was smooth. The subject was asked to stand erect with the shoulder, hips and heels touching the wall and with no footwear, heels together and looking straight ahead. The head was held comfortable erect, arms hanging loosely by the sides. A thin smooth scale was held on the top of the subjects head in the center, crushing the hair at the right angles to the tape and the height of the subject was read from the lower edge of the ruler to the nearest 0.1 cms.

### **C) Waist to hip ratio**

Waist and hip circumference were measured with the fiber glass tape. For waist circumference the subjects were made to stand facing the observer and then waist was measured at the right above the naval and the measurement with the abdomen in normal position i.e. neither it was inflated not pulled inside (Lohman 1998). For hip measurement, subject was made to stand sideways facing the observer and the maximal circumference of hip was taken (WHO 1995).

## **3 Dietary component**

The dietary information was elicited using a structured questionnaire. Dietary pattern for 3 days (which included two working and one non working day), consumption of MDM at school were also collected. The food behavior checklist (Annexure 3) was administered for 3 days to ascertain the trend. Frequencies for consumption of breakfast, lunch, vegetables and fruits were also looked into.



#### **4. Clinical signs and symptoms**

The children were examined for clinical signs and symptoms by the pediatrician of SSG hospital. Clinical signs and symptoms were studied for micronutrient deficiencies for iron, iodine and vitamin A deficiency using the WHO criteria. Information regarding common morbidities such as worm infestation, cough, cold, fever, headache and stomach ache was obtained as a part of morbidity profile using a reference period of 15 days (WHO, 1998).

#### **5 Biochemical parameters**

- **Hemoglobin**

Two Laboratory technicians trained to draw children's blood were taken to the schools for collection of blood sample. Disposable lancets were used. On the same day, the blood samples were sent for analysis to Thyrocare Laboratories, Vadodara. Hemoglobin was estimated by Cyanmet Hemoglobin method (INACG 2004).

##### **Principle**

On treating haemoglobin with Drabkin's reagent, haemoglobin present in blood reacts with potassium ferricyanide forms methaemoglobin and this compound is reduced by potassium cyanide to form cyanmethaemoglobin a rust colored compound, which is estimated spectrophotometrically at 540 nm.

##### **Standardization**

Cyanmethaemoglobin reference standard was obtained from "Qualigens Pvt Ltd". Spectrophotometer was calibrated using this method.

##### **Procedure**

Suitable aliquot of 0.75 ml, 2.25 ml and 3.75 ml haemoglobin standard was taken in separate test tubes and the volume was made up to 5ml by Drabkin's solution. In one test tube undiluted aliquot of 5 ml was taken as top standard. These were

read at 540 nm on a spectrophotometer after 30 minutes after adjusting the instrument to zero with blank solution (Drabkin's reagent). A factor for estimation of haemoglobin was calculated from the optical density obtained.

The estimation of haemoglobin was done according to the following steps:

- Any one finger of the hand was selected specifically the middle one. It was then wiped with a cotton swab dipped in ethanol and was allowed to dry.
- Then with a disposable lancet a bold prick was made.
- The first drop of blood was wiped off.
- Then a big drop of blood was allowed to form on the finger and then 20  $\mu$ l of blood was pipetted using a calibrated micropipette.
- The blood sample was added to 5 ml Drabkin's reagent and mixed thoroughly.
- This solution was allowed to stand (away from sunlight) for 30 minutes before being read on a spectrophotometer at 540 nm.
- Duplicate samples were collected from each subject.

## **PHASE II: UNDERSTANDING GROWTH DYNAMICS THROUGH LONGITUDINAL STUDY**

One of the objectives of the current study was to assess the growth pattern of school children. To achieve this objective, in addition to the cross sectional component of the study, a longitudinal component was also conducted

### **STUDY DESIGN**

Four schools were selected and finalized for this phase of the study. The school finalized for this phase was primarily based on the cooperation from the principal and agreeability for conducting the study for the next 3 years. Thus every year the school was visited in the month of July for data collection to ensure the completion of one year.

## **STUDY SAMPLE**

In the first year, all the children from 1<sup>st</sup> to 7<sup>th</sup> standard were enrolled for the study. Anthropometric measurement i.e. height and weight were recorded for all the children. In the first year, data was collected on 2282 children of which 1094 were girls and 1188 were boys. In the second year same children were followed up. Looking at the dropout rate and the pass out children of 7<sup>th</sup> standard on whom the data could not be collected, the sample size became 1555 children. In the third year, keeping the same criteria anthropometric data could be collected on 465 children; 227 boys and 238 girls.

## **PROCEDURE**

Height was measured by wall mounted fibre glass tape with the least count of 0.5 cms. Weight measurement was taken by standardized digital bathroom scale with the least count of 0.1Kgs. Both the equipments were standardized at regular interval.

A total of 465 children had 3 pair of data for consecutive 3 years. Paired data of these children were used for studying dynamics of growth and weight trends in the study population. The reference data used to identify the BMI cutoffs as well as conversion of weight and height to Z score were taken from CDC 2000 data set and WHO 2007 data set for growth parameters in children.

Age in months was used for converting BMI, weight and height to Z score as per CDC and WHO references. The cohort was divided into various sub groups for further analysis. Z score  $<-2$  SD for weight for age was considered underweight, Z score  $<-2$ SD for height for age was termed stunting and Z score  $<-2$ SD for BMI was termed as thinness.

### **PHASE III: IMPACT OF WEEKLY IRON FOLIC ACID (IFA) SUPPLEMENTATION ALONG WITH TWICE A YEAR DEWORMING TABLET ON GROWTH, HEMOGLOBIN STATUS AND PHYSICAL WORK CAPACITY OF SCHOOL CHILDREN**

For this phase, the intervention study was carried out using a randomized control trial. From the six schools, 3 schools were randomly selected and allotted in any one intervention group.

SCHOOL 1: Control Group – No Intervention – Standard Care was maintained

SCHOOL 2: Once weekly IFA+ Deworming twice a year

SCHOOL 3: Only Deworming twice a year

For IFA supplementation, 60 mg elemental iron + 0.5 mg Folic acid was given in the form of tablet. The supplementation was given for 30 weeks. For deworming, 400 mg Albendazole tablet was given twice a year.

This was a supervised trial and to monitor the regular consumption of the tablets, proper compliance sheets were maintained for all the 30 weeks. If a child was absent on the day of distribution of tablets, he/she would be given it on the next day. During the three week's Diwali vacation, each child was given 3 IFA tablet in a pouch to consume every week during vacation. After the vacation, the compliance for these tablets was also seen.

The pre - post data was collected on the children after 30 week intervention period. The long term impact of the intervention was also looked in the study. After a period of six months, where no intervention was given in any group, the data was again collected for growth parameter and haemoglobin status.

The time frame for the study in a nut shell is given in Table 3.3.

**Table 3.3: Time Frame of the Study**

<b>Details</b>	<b>Control Group</b>	<b>Experimental Group 1</b>	<b>Experimental Group 2</b>
Pre Data	June- July 2009	June- July 2009	June- July 2009
Intervention	Standard Care	IFA+DW	DW
IFA Tablets	-	August 2009 to February 2010 30 Weeks	-
Deworming		400 mg (July 2009) & 400 mg (January 2010)	400 mg (July 2009) & 400 mg (January 2010)
Post Data	15 <sup>th</sup> March 2010		
Washout Effect	September 2010		

The experimental plan for this phase is given in Figure 3.2

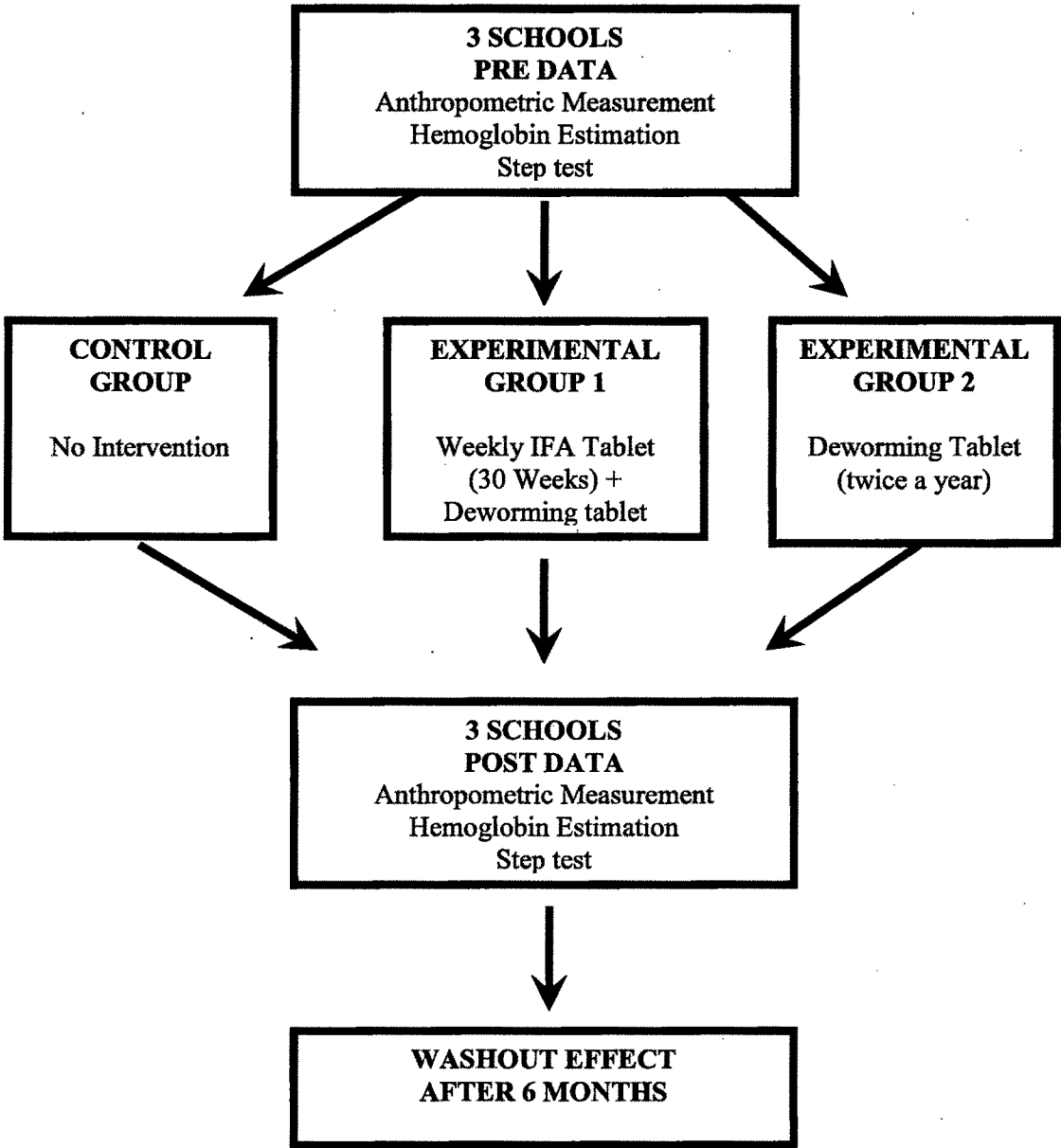
The following data was collected in this phase:

- Anthropometric Measurements (Height, Weight)
- Hemoglobin Estimation
- Step Test

The overview of sample size is given in Table 3.4. The number of children on whom data was collected for each of the above parameter is also given. The dropout rate for each parameter is also taken into consideration. The dropout rate for this phase of study ranged from 17 % to 40 %. This was because of high rate of absenteeism in the school. The dropout rate was high for hemoglobin estimation as it is an invasive method and as the children had given blood during pre data collection, some of them refused to give consent second time.

**FIGURE 3.2: EXPERIMENTAL PLAN**

**PHASE III: INTERVENTION STUDY**



**Table 3.4: Overview of Sample size for phase III**

Parameters	Control	IFA+DW	DW
<b>Anthropometry</b>			
Pre	210	322	195
Post	153	215	128
Drop out	<b>57(27.1)</b>	<b>107(33.2)</b>	<b>67(34.3)</b>
<b>Step Test</b>			
Pre	153	273	184
Post	131	191	153
Drop out	<b>22(14.3)</b>	<b>82(30.0)</b>	<b>31(16.8)</b>
<b>Hemoglobin</b>			
Pre	185	331	230
Post	108	230	161
Drop out	<b>77(41)</b>	<b>101(30.5)</b>	<b>69(30)</b>

Values in parenthesis indicate percentage

### **DATA COLLECTION**

The tools used to elicit the data collection are given below:

**Table 3.5: Tools used for data collection**

Indicator	Procedure
Weight	Digital Bathroom Scale
Height, waist, Hip	Fiber glass tape
Hemoglobin	Cyan met hemoglobin method
Step Test	Steps and Oximeter

## **METHODS**

**The methodology of data collection for anthropometry and hemoglobin estimation has been described in detail in the first phase of the study.**

### **Step Test**

An accurate way to assess fitness and physical work capacity is to complete a maximal aerobic test which records and measures the heart rate and oxygen consumption. There are many tests for assessing the aerobic capacity. One such test is the step test. Step test is based on heart rate recovery following a given work load. The pulse rate and the saturated peripheral oxygen readings were taken with the help of fingertip pulse oximeter p1. The make was ASPEN DIAGNOSTICS PVT Ltd. It is ISO 9001:2000 certified company. The oximeter gave the digital out put for saturated peripheral oxygen and pulse rate.

Step test was performed on all the children from 4<sup>th</sup> to 7<sup>th</sup> Standard to measure their physical work capacity. To perform this test, a 20 cm stool was designed for the children. The pulse rate per minute for each children was noted. The saturated peripheral oxygen reading was also taken. Then the children were administered the step test in which he/she had to step on the stool for 3 minutes. The numbers of steps were counted. After 3 minutes again the pulse rate and saturated peripheral oxygen reading was taken.

### **Principle of Pulse Oximeter**

A pulse oximeter measures and displays the pulse rate and the saturation of hemoglobin in arterial blood. This saturation of hemoglobin is a measure of the average amount of oxygen bound to each hemoglobin molecule. The absorption of visible light by a hemoglobin solution varies with oxygenation. The chemical binding of the different types of hemoglobin species changes the physical properties of the hemoglobin as well. The oxygen chemically combines with hemoglobin inside the red blood cells makes up nearly all of the oxygen present in the blood (there is also a very small amount which is dissolved in the plasma).



Oxygen saturation, which is often referred to as SaO<sub>2</sub> or SpO<sub>2</sub>, is defined as the ratio of oxyhemoglobin (HbO<sub>2</sub>) to the total concentration of hemoglobin present in the blood

$$SaO_2 = \frac{HbO_2}{(HbO_2 + Hb)}$$

Oxyhemoglobin (HbO<sub>2</sub>) and hemoglobin (Hb), have significantly different optical spectra in the wavelength range from 600nm to 1000nm, as shown in Figure 1

The P.O Pro will measure Arterial SaO<sub>2</sub> and express it as a percentage. Under normal physiological conditions arterial blood is 97% saturated, while venous blood is 75% saturated. The difference in absorption spectra of HbO<sub>2</sub> and Hb is used for the measurement of arterial oxygen saturation because the wavelength range between 600 nm and 1000nm is also the range for which there is least attenuation of light by body tissues (tissue and pigmentation absorb blue, green and yellow light and water absorbs the longer infra-red wavelength).

The half power spectral bandwidth of each LED is approximately 20-30nm. The LED's and photodiode chips are to be mounted on separate ceramic substrates. A small amount of clear epoxy resin will be applied over the LED's and photodiode for protection. Recessing and optically shielding the LED's and photodiode inside the sensor will minimize undesired specular light reflection from the surface of the skin and from the direct light path between the LED's and photodiode.

Oxygen saturation is divided into three ranges: normal saturation, high saturation, and hypoxic condition (low saturation level).

- High saturation (greater than 97.5%)
- Normal saturation (90 to 97.5%)
- Low saturation (less than 80%)

The normal pulse rate for humans ranges from 70 to 80 beats per minutes. The pulse oximeter is one of the most important advances in noninvasive monitoring because it provides a means of continuously and quickly assessing arterial blood oxygenation (Barker, 1987).

## **DATA ANALYSIS**

The data was entered into Microsoft excel spreadsheet and then subjected to appropriate statistical analysis using Microsoft excel data analysis package for calculating mean and standard deviation. The entered data of excel was imported into epi info 6 package and Z scores were derived by the CDC 2000 standards for undernutrition. Z scores by WHO 2007 standards were derived by Anthro plus package of WHO. The percent consumption of Mid Day Meal by children was arrived by the number of registered children for the month. 95 % confidence interval was calculated using formulas in the excel spread sheet. In the longitudinal phase of the study, anthropometric transitions were assessed by converting the corresponding parameter to Z score through epi info and WHO anthro package and their means were compared. Paired sample test was used for comparing individual group time transition. Chi square test was used for comparing weight transition among subgroups. Significance was assigned for a p value < 0.05. Nutrition trend analysis was done keeping the track of shift in individual nutritional status in 3 years. In the intervention phase apart from the anthropometric indices, F value were calculated to check the variation between the three intervention arms, while t test was used to check the significance level of intervention before and after the study period.