CHAPTER – II
MATERIALS AND METHODS

1. STUDY AREA:

MIDC Shiroli is one of the industrial areas near Kolhapur city. It is located six
kilometer away from city. There are fifty foundries present in Kolhapur, in which
about 9000 workers are working. In India near about 6000 foundries are present.
In foundry variety of foundry process are carried out which includes making the
pattern, preparation and mixing of sand, making and assembling the mould,
melting metal in furnace and finally removing all adherent sand and superfluous
metal from the finished casting. Workers are involved in these different activities.
The environmental conditions are hot, noisy and dusty. The overall occupational
environment inside the foundry in different sections affects the physiological
behavior of workers. Mostly silica dust, coal dust and metal dust produced in
foundry operations affects the respiratory system of workers.

2. SELECTION OF ANIMAL MODEL:

In our previous study by Sawant and More (2003). it was found that, more
than 90% of foundry workers had respiratory impairment and the complaints
regarding warm body, body ache, chest pain, weakness, lower back pain, eye
irritation, problem in vision (foggy vision), pain in the joint of hands and legs
some workers also suffers from physical injury like burns from splashes of molten
metal and hand injuries due to manual handling of metal materials. A study of
lung function shows that, there was significant decease in peak expiratory flow
rate, tidal volume, total lung volume and forced expiratory volume in one second.
The response of rat to inhalation to silica dust is dependent upon weight of animal. The increase in breathing frequency was greatest in smaller animals. This may reflect a greater effective dose of stimulating agent per lung surface area in smaller animals.

Initially for the determination of exposure period three rats were kept for in the foundry environment, but due to higher concentration of dust and other stress factors all rats died after 25 hours. To study physiological reactions to foundry dust exposure, albino rats (Rattus norvegius) were exposed for period of 8 hrs, 16 hrs and 24 hrs respectively in the foundry environment. The physiological reactions of rat were similar to those of human. Therefore, the rat animal model was preferred and used to investigate the mechanism of response and to identify etiologic agents related to foundry dust exposure.

METHODS


The male albino rats (Rattus norvegicus) weighting about 220 to 240 gms were selected as experimental animals. Rats were divided into two groups control and experimental (n = 3). Experimental groups of animals were divided into five sets for each section of foundry and exposed for 8 hrs, 16 hrs and 24 hrs respectively inside the foundry in five different sections during exposure the rats fed with Gold Mohair Rat Feed (Hindustan Lever Ltd. Mumbai) and water ad libitum. The control groups had three rats which were kept in laboratory environment. Experimentations were conducted according to “INSA – Ethical guidelines for use of animals in scientific research.”
2. Environmental monitoring of workplace

In the iron foundry, thousand of workers were working in various sections like sand plant, core shop, moulding section, furnace section and fettling shop. Thousand of foundry workers attended the foundry units at least 8-10 hours of the day. In the foundry series of processes took place and the work was mechanized. Many times worker carried same task all throughout day and on every working day of year. The worker did only the job that he has been given to do in exchange he received a wage in cash.

The work place study shows that higher concentration of dust, high intensity of noise, radiant heat near the furnace and vibrations are the main factors, which make the working environment stressful for workers.

In the foundry work place study was carried out by different methods

i) **Sound Measurement**: Sound level of different sections in foundry unit was recorded by sound level meter. The sound level was recorded in decibels (db).

ii) **Heat**: Recording thermal data such as dry bulb thermometer and digital thermometer was made in foundry sections during working hours. Assessment of thermal load with special reference to different physiological responses of foundry workers was carried out.

iii) **Light (Illumination)**: The illumination levels at different section inside the foundry unite were recorded by a lux meter. The illumination level recorded in lux.
iv) Foundry dust:

Study of foundry dust by sampler.

Working environment in the foundry is dusty. Health risk factors in foundry work are metal dust, silica dust, coal dust, metal fumes, and various products produced during combustion and heat decomposition, chemical products relating to resin compounds and physical factors such as noise, vibrations and high temperature. Out of these factors, most problematic factor for foundry workers is dust containing quartz, leading to silicosis. Lung cancer has increased in foundry workers. Time of exposure to high concentrations, silicosis progresses fast even in young workers who are in their 20s and 30s exposed to silica within 5 year period for this we measured the level of respiratory dust in foundry workplaces and analyzed the trace elements. We have measured the dust concentration by using Respirable Dust Sampler Model RDS-3 Mfd. By Spectra lab- Thane, (M.S.).

Method:

Measurement of the concentration of the dust particles in the work environment can be made by monitoring in the worker’s respiratory zone by fixing sampling unit to the worker’s clothing which is called as personal sampling.

For enabling valid integrated samples of the air actually inhaled by the worker, under normal working conditions. The personal sampler is very useful, and is recommended by the National Institute of Occupational Safety and Health (NIOSH) and Occupational Safety and Health Administration U. S. A.

Dust sampling in the foundry was done with Respirable Dust Sampler Model RDS-3, Mfd. By Spectra lab, Thane (M. S.). This instrument consists of pump,
the sampling head (Filter paper holder and cyclone), battery and tygon tubing etc.

The Pump: - It is lightweight with rechargeable batteries lasting for 8 hours having voltage 4.8 V with aluminum body weight is about 0.8 kg. It is provided with Rota meter to adjust the flow rate.

The Sampling head (Filter paper holder and cyclone):- It consists of 47 mm diameter holder for 0.4µ filter. The cyclone is designed to reject particles ≤ 10µ. Particles between 0.4 to 10 µ are collected on the filter paper. Filter paper holders are made up of Acrylic Body. Whose inner diameter is 47 mm.

Tygon tubing: It connects the sampling pump to cyclone. The tubing is long enough so the pump can be worned on the worker’s waist and cyclone can be mounted on the worker’s collar.

Initially filter paper was selected as Whatman No. 1, with 47 mm diameter. Paper was kept in oven at 20°C for two hours and after that filter paper kept at desicator. Before sampling weight of filter papers were taken in gms. Then filter paper was fitted in filter paper holder and the flow rate is adjusted to 3 LPM (3 Lit/min) sampling was done for 1 hour, the final LPM was checked and if there was any change average LPM was considered for volume calculation. After sampling filter paper was removed from the cyclone and weighted. The cyclone was constructed in such way that when air enters due to centrifugal force, particles heavier than 10u size are thrown out to the bottom. The cyclone and filter paper holder are connected together in such way that air enters the cyclone, heavy particles travels through the filter paper. Particles between 0.4u and 10u are collected on the filter paper. The filter paper was weighted before and after the sampling keeping flow rate 3 LPM, sampling was carried out for one hour.
**Sampling procedure:**

i) Workers were selected to be sampled and explained purpose of sampling was to monitor the workers exposure to dust.

II) Also explained the worker how the cyclone works and to the tip the cyclone works and not to the tip the upside down, while sampling.

III) Sampler pump (filter paper holder cyclone) was attached to waist region by using straps.

IV) Cyclone and filter paper holder was attached to workers collar. This should be within workers breathing zone.

V) Explained the workers that the sampler inlet must face away from clothing, don’t cover it with clothing.

VI) Sampler was checked for

   a) pump was still running

   b) Inlet and sampler in correct position

   c) Worker was working in same section.

vii) After sampling sampler was collected sample run time was noted in minutes. And post calibrations performed i.e. filter paper was removed carefully from holder weighted in laboratory and final weight was noted and performed calculation.
CALCULATIONS:

Volume of air sampled was calculated by following formula

\[ \text{PM}_{2.5} = \frac{W}{V} \]

\[ W = W_2 - W_1 \]

\[ W_1 = \text{Initial weight of filter paper} \]

\[ W_2 = \text{Final weight of filter paper} \]

\[ V = \text{Volume of air sampled} = R \times T \]

\[ R = \text{Sampling rate} = 3 \text{ LPM} \]

\[ T = \text{Sampling time} = 60 \text{ min.} \]

\[ V = R \times T \]

\[ = 3 \times 60 = 10 \text{ Lit.} \]

\[ = 180 \times 10^{-3} \text{ (conversion into Cubic Meter)} \]

\[ \text{PM}_{2.5} \text{in} \mu g/m^3 = \frac{W}{V} \]

Analysis of Trace Elements from Foundry Dust

Dust sample preparation was done for chemical analysis. The filter paper was subjected to hot acid extraction to put metals in solution. The filter strips were placed in a beaker and extracted by refluxing on a hot plate using 10 ml HCL (8%) solution. The digestate was filtered before analysis (USEPA 1999b)
Analysis of trace elements was carried out by Atomic Absorption Spectrophotoscopy.

3. BEHAVIORAL STUDY

Experimental animals i.e. rat were kept in cages and exposed inside different foundry sections. During exposure time various activities of animals were observed. The behavioral changes in animals were studied by Animals Activity Monitor (Made By Electron Engineering Corporation, Madras 41). The monitoring of animal behavior involves placing the animal in a chamber designed to automatically record the gross motor behavioral activities. Different types of activities of animal monitored by recording vibrations caused by movement of animal. The sensors located at the base plate detect vibrations in the form of beeps and the beeps on low sensitivity were recorded by counter.

4. PHYSIOLOGICAL STUDY

After exposure of experimental animals in different sections of foundry for period of 8 hrs, 16 hrs and 24 hrs respectively, these animals were sacrificed by cervical dislocation and following study was carried out.

a. Nasal Lavage Study

After cervical dislocation immediately sterile physiological saline (0.9 % Nacl) were instilled 2ml. in each nasal cavity using Pasteur’s pipette. The saline was held inside nasal chamber for 10 sec. and then reabsorbed by Pasteur pipette. The smear of the lavage was prepared.
b) **Bronchoalveolar lavage study:**

After cervical dislocation, animal cut open, chest cavity exposed and trachea incision was made, through that with the help of Pasteur’s pipette 5 ml. sterile saline instilled through trachea. The saline was kept inside for 15 sec. and immediately reabsorbed by Pasteur’s pipette. Check the volume of saline, then smear of bronchialveolar lavage was prepared on slide, air dried and fix in methanol. The slides were stained and observed under microscope.

Microphotographs of nasal lavage smear and bronchoalveolar smear were taken by using Digital Camera of Nikon.

iii) **Histological Technique for Trachea, Lung, Kidney, Liver and Adrenal:**

The experimental animals after exposure inside the different sections of foundry for appropriate time sacrificed by cervical dislocation. The animals cut open, trachea, lung, adrenal, kidney and liver tissues were fixed in 20 % CAF (2 gm. Calcium acetate in 10 % Formalin). The tissues were dehydrated and paraffin blocks were prepared. The sections were fixed on glass micro slides by using albumen as adhesive.

A. **Staining by Haematoxylin- Eosin (HE):**

1. The sections were deparaffinised in xylene and were brought to distilled water by passing Alcohol grades. Absolute Alcohol→ 90%
   Alcohol→ 70% Alcohol→ 50% Alcohol→ 50% Alcohol →30% Alcohol→ Distilled water for 10 minutes each.

2. Then sections were stained in Haematoxylin for four to five minutes.
3. Differentiated in distilled water.

4. Dehydrated through 30% alcohol → 50% alcohol → 70% alcohol for 1-2 minutes each.

5. Stained in alcoholic eosin for 2 min.

6. Differentiate in 70% alcohol.

7. Quickly dehydrated through 90% and absolute alcohol.

8. Cleared in xylene and mounted in DPX.

iv) Hematology:

After exposure, the experimental rats to different sections of iron foundry for appropriate time (8hrs, 16hrs, 24hrs, respectively). Animals were sacrificed by cervical dislocation and blood samples were collected with syringe by cardiac Puncture, in EDTA bulb used for Haemoglobin concentration, RBC count, WBC count, and Platelet count.

a) Hemoglobin Concentration:

The percentage of hemoglobin was determined by using Sahli’s Hemometer as described by Wright (1966). Readings were taken in triplicate for accuracy and recorded in gms / 100 ml. of blood.

b) Total RBC Count:

Enumeration of Red Blood Corpuscles was done by Neubauer’s counting chamber. The blood sample diluted by RBC diluting fluid in RBC pipette and the fill the Neubauer’s chamber. The haemocytometer placed under microscope and
red blood cells were counted in RBC squares in triplicate under high power of microscope.

c) **Total WBC count:**

Total White Blood Corpuscles count was done by Neubauer’s counting chamber. The haemocytometer was placed under microscope. All readings were taken in triplicate for accuracy.

d) **Differential count:**

A thin blood smear was prepared on glass slide by fresh blood, dried, fixed in methanol and stained with Leishman’s stain. The smear was examined under high power of microscope for differential count. Microphotographs of blood smear were taken by Digital Camera of Nikon.

e) **Total Platelet Count:**

The platelet count was done by using Neubauer’s Counting Chamber. 0.02 ml blood sample was sucked by micropipette and diluted in 2 ml. of platelet diluting fluid, mix well and charge the drop in counting chamber. Keep the chamber for 2-3 minute to settle the platelets. Then counting was taken on four WBC squares under high power of microscope. The readings were taken in triplicate for accuracy.

7. **Serology Methods:**

The blood samples of different experimental animals were collected in plain bottle and centrifused at 3000 rpm. for 15 minutes. The separated serum taken out by dropper in another clean test tube and stored at room temperature. Serum is suitably diluted with normal physiological saline (0.9% Nacl) as one part serum
and 5 part saline. The serum was analyzed on Auto-analyzer “Merck Microlab 200” (E. Merck India Ltd., Mumbai). The kits used for analysis of serum were purchased from E. Merck India Ltd. Mumbai. The diluted serum was analyzed for liver function tests, creatine phosphokinase (CPK), creatine kinase muscle and brain units (CKMB).

Liver Function Test:

I. Total and Direct Bilirubin:


   Kits used: Diagnostica MERCK -0499

Principle:

Sulfanilic acid reacts with sodium nitrite (NaNo₂) to form diazotized sulfanilic acid. In the presence of dimethyl sulfoxide, conjugated and unconjugated bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. In the absence of diethyl sufoxide, only the conjugated bilirubin reacts to give azobilirubin.

Sulfanilic acid + NaNo₂ → Diazotized Sulfanilic acid

Bilirubin + Diazotized Sulfanilic acid → Azobilirubin
Procedure:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagents</th>
<th>Blank</th>
<th>Total Bilirubin (T)</th>
<th>Direct Bilirunin (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diazo A</td>
<td>100µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>2</td>
<td>Diazo B</td>
<td>-</td>
<td>10µl</td>
<td>10µl</td>
</tr>
<tr>
<td>3</td>
<td>Activator</td>
<td>100µl</td>
<td>100µl</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water</td>
<td>250µl</td>
<td>250µl</td>
<td>250µl</td>
</tr>
<tr>
<td>5</td>
<td>Serum</td>
<td>-</td>
<td>20µl</td>
<td>20µl</td>
</tr>
</tbody>
</table>

Mix reagents will keep the test tubes in the dark at room temperature for five minutes to develop purple colored complex. Then measure within 30 min. on filter 546. For standard take 4 ml. of artificial standard in cuvet and measure on filter 546 on kinetic mode of Auto analyzer and calculated as gms/ dl.

II. Total Serum Proteins:

Method: Modified by Biuret and Doumas (1971)

Kits used: Diagnostic MERCK- 0499.

Principle:

Colorimetric determination of total protein based on the principle of Biuret reaction (Copper Salt in an alkaline medium), Protein in plasma or serum sample
forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of blue color is proportional to the protein concentration.

**Procedure:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solution</th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent</td>
<td>1ml.</td>
<td>1ml.</td>
<td>1ml.</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>20µl.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>-</td>
<td>20µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mixed well and incubate for 10 min at $37^\circ$C to develop purple color complex which is measured on filter 546 nm. on Kinetic mode of auto analyzer and calculated Proteins as gms. / dl.

**III. Albumin:**

Method: Modified Doumasa *et al.*, (1971)

Kits used: Diagnostica MERCK- 0499

**Principle:**

The reaction between albumin from serum or plasma and the dye bromocresol - green produces a change in color that is proportional to the albumin concentration, which is measured at 546nm.


**Procedure:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solution</th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent</td>
<td>1ml.</td>
<td>1ml.</td>
<td>1ml</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>10µl.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and incubate for 10 min at 37°C and measured on 546 nm on Kinetic mode of auto analyzer and calculated as gms./dl.

**IV. Globulins:**

To obtain the amount of globulins present in the serum sample, the amount of serum albumin estimated was subtracted from the total proteins estimated.

\[
\text{Total Protein - Albumin} = \text{Globulins.}
\]

**V. Alkaline Phosphatase (U/L):**

Method: Modified Bessey - Lowry Brock (1946)

Kit used: Diagnostica MERCK- 0296

**Principle:**

Alkaline phosphates catalyses the hydrolysis of p- Nitro phenyl phosphate to yellow colored P-nitrophenol and phosphate Kinetic determination of Alkaline Phosphates based upon following reactions.
P- Nitro phenyl phosphate + H₂O → p - Nitrophenol + Inorganic phosphate
ALP = Alkaline phosphatase

The rate of increase in P- nitro phenol is determined and it is directly proportional to the alkaline phosphates activity in the serum.

Procedure:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solution</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent</td>
<td>400µl</td>
<td>400µl</td>
<td>400µl</td>
</tr>
<tr>
<td>2</td>
<td>Start Reagent</td>
<td>100µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>3</td>
<td>serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Standard</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix properly and incubate for 1 min. at 37°C and measure on filter 405 nm on Kinetic mode of Analyzer.

B. OTHER SERUM ENZYME ANALYSIS:

I. Creatine Phosphokinase (CPK) U/L:


Kit used : Diagnostica MERCK- 0696.

Principle : The creatine phosphokinase is coupled to the pyruvate kinase reaction. The pyruvate formed is measured at 440nm through the formation of the bydrazone with 2-4 Dianitrophenylhydride.

Creatine

ATP + Creatine phosphokinase → ADP + Creatine phosphate
**Procedure:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solution</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent</td>
<td>400µl</td>
<td>400µl</td>
<td>400µl</td>
</tr>
<tr>
<td>2</td>
<td>Start reagent</td>
<td>100µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>20µl</td>
</tr>
<tr>
<td>4</td>
<td>standard</td>
<td>-</td>
<td>20µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and incubate for about 1 minute at room temperature and measure on filter 340 nm at 37°C on U.V. Kinetic mode of auto analyzer.

II. Creatine Kinase Muscle and Brain Unit (CKMB) U/L:


Kits used: Diagnostica MERCK- 0696

**Principle:**

The creatine Kinase (CK) is dimeric molecule composed of ‘M’ (muscle) and ‘B’ (brain- nerve cells) subunits, which are immunologically distinct. It exists as three main isoenzymes CK-MM, CK-MB. The CK-MM is found in the muscle, while CK-MB is found mainly in the myocardial cells. The CK- BB is found, mainly in the brain and lungs and enters the blood stream. ‘M’ subunits of CKMM and CKMB are inactivated by reaction with anti ‘M’ antibody (immunoglobulin). The remaining subunit is measured enzymatically.
Procedure:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solution</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent</td>
<td>400µl</td>
<td>400µl</td>
<td>400µl</td>
</tr>
<tr>
<td>2</td>
<td>Start reagent</td>
<td>100µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>20µl</td>
</tr>
<tr>
<td>4</td>
<td>Standard</td>
<td>-</td>
<td>20µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and incubate for about 1 minute at room temperature and measure on filter 340 nm at 37°C on kinetic mode of auto analyzer.

III. Serum Aspartate Transaminase/ Serum Glutamate oxaloacetate Transaminase (SGOT)

Quantitative estimation of SGOT


Kit used: Diagnostic reagent Kit. Code No. - 25913.

Principle: $\text{SGOT}$ catalyses the following reaction.

$$\text{a-Ketoglutarate} + \text{L-Aspartate} \rightarrow \text{L-Glutamate} + \text{oxaloacetate}$$

Oxaloacetate which is formed get coupled with 2, 4 - Dinitrophenyl hydrazine (2, 4 - DNPH) to give the corresponding hydrazones, which gives brown color in alkaline medium and this is measured calorimetrically.

Sample: 0.1 ml serum.

Reagents:

1. Reagent 1 - Buffered Aspartate-α- KG Substrate, pH 7.4
2. Reagent 2 - DNPH color reagent
3. Reagent 3 - Sodium Hydroxide, 4N.
4. Reagent 4 - Working pyruvate Standard 2 mm.
Procedure:

Standard curve:

<table>
<thead>
<tr>
<th>Test Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activity (units/mL)</td>
<td>0</td>
<td>24</td>
<td>61</td>
<td>114</td>
<td>190</td>
</tr>
<tr>
<td>Reagent 1: Buffered Aspartate-α-KG Substrate, pH 7.4 ml</td>
<td>0.5</td>
<td>0.45</td>
<td>.40</td>
<td>0.35</td>
<td>0.3</td>
</tr>
<tr>
<td>Reagent 4: Working Pyruvate Standard, 2 mM</td>
<td>-</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>Distilled water, ml.</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Reagent 2: DNPH color reagent, ml.</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mixed well and allowed to stand at room temperature for 20 minutes.

Solution 1, ml 5.0 5.0 5.0 5.0 5.0

Mixed well by inversion. Allowed to stand at room temperature for 10 minutes and measured against distilled water at 505 nm on kinetic mode of auto analyzer. Standard graph was plotted by taking enzyme activity on X-axis and O.D. on Y-axis.

Test:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagent</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent 1: Buffered Aspartate-α-KG Substrate, pH 7.4 Incubated at 37°C for 5 minutes</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Serum Mixed and incubated at 37°C for 60 minutes</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>3</td>
<td>Reagent 2: DNPH color reagent</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>
Mixed well and allowed to stand at room temperature for 10 minutes and read against distilled water at 505 nm on Kinetic mode of multianalyzer 'Express Plus', Mfd. Chiron Diagnostics, USA.

**Calculations:**

Marked the O.D. of Test (T) on ‘Y’ axis of the standard curve and extrapolated it to the corresponding enzyme activity on the X- axis.

IV. Alnine Transaminase/Serum glutamate pyruvate Transaminase (SGPT) /Quantitative estimation of SGPT

**Method:** Modified Rittman and Franked (1966).

**Kits used:** Diagnostic test kit, code No. 25912.

**Principle:**

Kinetic determination of SGPT activity based on following reaction.

\[
\text{ALT} \quad \text{L- Alanine + } \alpha\text{- Ketoglutarate} \rightarrow \text{L- Glutamate + Pyruvate.}
\]

ALT: Alanine amino transferas

Pyruvate so formed is coupled with 2,4- Dinitrophenyl hydrazine, which gives brown color in alkaline medium and this can be measured calorimetrically.

Sample: 0.1 ml serum

**Reagents:**

1. Reagent 1: Buffered Alanine- \( \alpha \)- KG Substrate, pH 7.4
2. Reagent 2: DNPH color reagent
3. Reagent 3: Sodium Hydroxide, 4N.
4. Reagent 4: working Pyruvate standard, 2mM.
**Procedure:**

**Standard curve**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enzyme activity (units/ml)</td>
<td>0</td>
<td>28</td>
<td>57</td>
<td>97</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>Reagent 1: Buffered Substrate, pH 7.4 ml</td>
<td>0.5</td>
<td>0.45</td>
<td>0.4</td>
<td>0.35</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Reagent 4: Working Pyruvate Standard, 2 mM, ml</td>
<td>-</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water, ml.</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>Reagent 2: DNPH color reagent, ml.</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Mixed well and allowed to stand at room temperature for 20 minutes</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>Solution 1, ml</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Mixed well by inversion. Allowed to stand at room temperature for 10 minutes and measured at 505 nm on Kinetic mode of multianalyzer ‘Express Plus’, Mfd, Chiron Diagnostics, USA.

Plotted a standard graph by taking enzyme activity on X-axis and O.D. on Y-axis.
**Test:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagent</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent1: Buffered substrate, pH 7.4 Incubated at 37(^\circ)c for 5 minutes</td>
<td>0.5ml</td>
</tr>
<tr>
<td>2</td>
<td>Serum/ Mixed well and incubated at 37(^\circ)c for 30 minutes</td>
<td>0.1ml</td>
</tr>
<tr>
<td>3</td>
<td>Reagent 2: DNPH color reagent Mixed well and allowed to stand at room temperature for 20 minutes</td>
<td>0.5ml</td>
</tr>
<tr>
<td>4</td>
<td>Solution 1</td>
<td>0.5ml</td>
</tr>
</tbody>
</table>

Mixed well and allowed to stand at room temperature for 10 minutes and read at 505 nm on Kinetic mode of multi analyzer.

**Calculations:**

Marked the OD of test on the Y-axis of the standard curve and extrapolated it to the corresponding enzyme activity on the X-axis.