The present study was started after obtaining the ethical clearance from the institutional ethical committee vide their letter Ref.no. SMC/EC/2010/87 dated: 27/11/2010 (Annexure 1). A, “Data collection format” (DCF) was designed (Annexure 3). Patients attending out and in-patient Departments of Tuberculosis and Respiratory Diseases of C.S.S Hospital were enrolled in the present study. Informed consent was taken in each case (copy of specimen format enclosed Annexure no. 2). The study was conducted in the department of Biochemistry, Subharti Medical College, Meerut.

General information of the patients including name, husband/father’s name, age, sex, marital status, occupation, address, contact number were asked and recorded in each case.

Presenting complaints were noted on DCF. Detailed present, past and family history was taken and recorded. Specific history in relation to respiratory diseases like fever and its evening rise pattern, loss of appetite, shortness of breath, dull ache, cough and expectoration were noted.

In general examination of the patients; temperature, pulse, b.p., height/weight, respiration rate, icterus, clubbing, oedema, cyanosis and any obvious lymphadenopathy were recorded. Systemic examination of central nervous system, cardiovascular system, abdomen and respiratory system were performed and positive findings noted.

During respiratory examination the signs like diminished movements, dullness on percussion, and absent breath sounds were specifically noted.

Routine investigation like Hb, TLC/DLC, ESR, GBP, CBC, Chest X-ray PA & Lateral views were done in all the cases on the basis of radiological finding. Patients were confirmed to be having pleural effusion. Ultrasound, ECG and some specialized test were performed in different patients to confirm the diagnosis.
General examination of a patient

Plate 1: Lymphadenopathy

Systemic Examination of Patient

Plate 2: Auscultation
Respiratory Examination of Patient

Plate 3: Percussion

Slide of Pleural Fluid
Plate 4: Lymphocytes

**Following diagnostic criteria was adopted for the diagnosis of the patients:**

1. Patients with enlarged cardiac shadow in plain X-ray chest with clinical or echocardiographic (ECG) evidence of cardiac dysfunction, with one or more of the mentioned alterations: pulmonary venous congestion on radiography, peripheral edema, tachycardia, or ventricular gallop were diagnosed as Congestive heart failure (CHF).

2. Patients having raised urea and creatinine levels in the presence of clinical evidence of fluid overload (e.g., pulmonary or peripheral edema) and an absence of malignancy or respiratory infections were diagnosed as chronic renal failure.

3. The presence of clinically and radiologically confirmed pneumonia with no direct or indirect evidence of bacterial presence suggested as Parapneumonic effusion.

4. Patients with pneumonia along with one or more of the following indicators of bacterial invasion of the effusion: presence of pus, bacteria in Gram’s stain smear or culture, and pH under 7.0 or progressively decreasing to less than 7.20 was suggested as Empyema.

5. The presence of relevant auto-antibodies and clinical signs were aided in the diagnosis of Collagen vascular disease.

6. Pancreatitis was suspected as chronic alcoholics, with history of severe deep boring abdominal pain and dys-electrolytemia following a bout of heavy alcohol intake, with relevant findings on abdominal ultrasound and raised serum amylase levels.

**X-ray**
Plate 5: Para-pneumonic effusion

Plate 6: Empyma
Plate 7: Chest X-ray with Right Pleural Effusion

Plate 8: Chest X-ray with Left Pleural Effusion

7. Patients with history of smoking, persistent cough, x-ray findings, cytological diagnosis of the pleural or broncho alveolar lavage (BAL) and the elevated serum levels of specific tumor marker antigens were diagnosed as malignant pleural effusion.

8. Presence of the first or any two of the other criteria must be present to label a case as tubercular;
   i) Bacterial confirmation of the presence of mycobacterium tuberculosis (Direct smear or culture or histological findings in tissue sample or fluids),
   ii) FNAC or tissue biopsy with histopathological findings suggested of tuberculosis,
iii) radiological findings suggestive of tuberculosis, iv) definitive clinical improvement within 2 months of anti-tubercular therapy and v) a history of contact with open cases of tuberculosis and a positive reaction >20 mm induration to 5 tuberculin unit (5TU) purified protein antigen was the adopted criteria.

Plate 9: Bronchoscope instrument
Plate 10: BAL (Broncho alveolar lavage)

Plate 11: Endoscopic view of carina by Bronchoscopy

Plate 12: Culture of Mycobacterium tuberculosis in LJ medium
Plate 13: FNAC

Plate 14: Mantoux Test/Purified Protein Derivative Test (PPD)
Additional investigations were done to confirm/access the extent of the disease:

<table>
<thead>
<tr>
<th>s. no.</th>
<th>Investigations</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Echocardiography, X-ray Chest</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>2</td>
<td>USG abdomen, Blood Urea, Serum Creatinine, FNAC, Pleural biopsy, CT, MRI</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>3</td>
<td>X-ray chest, CT, Gram Stain/ZN staining of Expectorant/pleural fluid</td>
<td>Paraneumonic effusion</td>
</tr>
<tr>
<td>4</td>
<td>X-ray chest, Bronchoscopy, Cytology, Gram Stain/ZN staining of Expectorant/pleural fluid /BAL</td>
<td>Empyema</td>
</tr>
<tr>
<td>5</td>
<td>Antibody detection by ELISA</td>
<td>Collagen vascular disease</td>
</tr>
<tr>
<td>6</td>
<td>Serum amylase, test for electrolytes (Na⁺/K⁺/HCO₃⁻) imbalance, USG abdomen,</td>
<td>Pancreatitis</td>
</tr>
<tr>
<td>7</td>
<td>Total and differential leucocyte counts, X-ray / USG affected part, FNAC / biopsy of suspected lesion, Bronchoscopy and BAL examination, Cancer marker</td>
<td>Malignancy</td>
</tr>
<tr>
<td>8</td>
<td>Pleural fluid/sputum smear examination for AFB by Zeihl-Neelsen Staining, Mycobacterial Culture of pleural fluid/sputum by using liquid culture media/BACTEC system, X-ray of chest, USG of affected part, Pleural biopsy, Montoux test, ADA estimation</td>
<td>Tuberculosis</td>
</tr>
</tbody>
</table>
There after patients were subjected to pleural tapping.

**Pleural fluid aspiration**

Seat the patient on a stool leaning forward over the side of the bed, with arms folded under the chin. The site was chosen carefully, taking account of the likely position of the diaphragm and the top of the effusion as judged by percussion. Simple M-mode ultrasound was very helpful in aspirating loculated effusion. Local anaesthetic was infiltrated intradermally, subcutaneously and into the muscle and parietal pleura, infiltration continuing until fluid is aspirated. Care should be taken to insert the needle gracing the upper margin of the rib, to avoid damage to inter-costal nerve and vessels, which lie immediately below each rib. After finding the fluid, about 5ml of fluid was taken for biochemical and histopathological examination, and another 5ml of fluid was taken for ADA estimation, in two plain glass vials with cap. Turbid and hemorrhagic pleural fluids were excluded. The samples for ADA estimation were processed immediately.
Plate 15: Pleural fluid aspiration

The entire process was performed under sterile condition.

270 patients of confirmed pleural effusion were finally enrolled for the present study. Pleural fluid microscopy and differential count was done and finally the patients who have lymphocyte count more than 50% (lymphocyte rich), were included in the study and these were 108.

In these selected patients, the pleural fluids were tested for pH, glucose, proteins, LDH levels, total ADA, cytology and microbial testing. Direct sputum smear examination was done to confirm the diagnosis. These patients also underwent blood investigations for total protein, random blood glucose and other relevant tests as mentioned above.

Based on the above mentioned diagnostic criteria, patients were clubbed into two groups. One as tubercular and other as non-tubercular, both with lymphocytic rich pleural effusion.

All the data were recorded and statistically analyzed using ANOVA test.
Plate 16: Vitros 250 Autoanalyzer

Sputum sample collection and examination:

Collection: Two samples were collected from PTB suspect as per the criteria laid down by RNTCP (Revised National Tuberculosis Control Program). Smear was made and examined under microscope after ZN stain.

Sputum smears is done for acid-fast bacilli. The preferred method is fluorescence microscopy, which is more sensitive than conventional Ziehl-Neelsen (ZN) staining.

Fix the smear on the slide

↓

Cover the fixed smear with carbol fuchsin for 3 minutes

↓

Heat, rinse with tap water and decolourise with acid alcohol for 3-5 seconds

↓

Counter stain with methylated blue for 30 seconds
Rinse again with tap water

Observe under the microscope

The bacilli appear as red, beaded rods, 2-4 µm long and 0.2-0.5 µm wide. (Use the oil immersion lens (100) and 6 or 8 eye-piece lens)

<table>
<thead>
<tr>
<th>Smear grading-Number of bacilli</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB per 100 oil immersion fields</td>
<td>Negative</td>
</tr>
<tr>
<td>1-9 AFB per 100 oil immersion fields</td>
<td>Scanty</td>
</tr>
<tr>
<td>10-99 AFB per 100 oil immersion fields</td>
<td>+ (1+)</td>
</tr>
<tr>
<td>1-10 AFB per 100 oil immersion fields</td>
<td>++ (2+)</td>
</tr>
<tr>
<td>&gt;10 AFB per 100 oil immersion fields</td>
<td>+++ (3+)</td>
</tr>
</tbody>
</table>

**BIOCHEMICAL PARAMETERS - Protocols**

**Hb**

Determined by B-Cyanmethaemoglobin Method (Colorimetric method)

**Principle:**

Hb is oxidized to methaemoglobin by potassium ferricyanide. MetHb in turn is converted to a stable compound cyanmethaemoglobin by potassium cyanide. Color of this solution is compared against a standard of known Hb value colorimeter. The blood is diluted in Drabkin’s dilution fluid, which hemolyses the red cells, converting the hemoglobin into cyanmethaemoglobin. The solution obtained is examined in a spectrophotometer or colorimeter.

**Reference range:** 14-18 g/dl: males 12-16 g/dl: females
DLC

By manual method:-

Principle:

Differential leucocyte count is made by visual examination of blood film prepared on slide by spread or wedge technique & stained by Leishman stain.

Counting the cells under the microscope using 40x lens in a strip running the whole length of the film. One or more strips counted until at least 100 cells counted.

Result:- it is expressed as percentage of each type of cell.

Neutrophils – 45-68%

Lymphocytes- 20-40%

Eosinophils- 0-8%

TLC

Manual Method:-

Principle: When blood is mixed with WBC diluting fluid, glacial acetic acid of the fluid lyse the RBCs & gentian violet stains WBC nuclei deep violet black.

Equipments:

1. WBC pipette/glass tube & pipette
2. Improved Neubauer chamber
3. WBC diluting fluid (TURK’S fluid)
- Glacial acetic acid
- Sodium chloride

1% aqueous solution of gentian violet & distilled water.

This uses the hemocytometer and bulk or bulb dilution of blood.

**Calculations:**

No of WBC’s in 4 large squares = \( N \)

Volume of 4 squares = \( 1 \times 1 \times 1 / 10 \times 4 \) cumm = \( 2/5 \) cumm

Dilution factor-20

\( 2/5 \) cumm blood contains = \( N \times 20 \)

Therefore 1 cumm blood contains = \( N \times 20 \times 5/2 = N \times 50 \)

**ESR:**

The **ESR** is a simple non-specific screening test that indirectly measures the presence of inflammation in the body. It reflects the tendency of red blood cells to settle more rapidly in the face of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins. Changes in red cell shape or numbers may also affect the ESR.

The **Westergren method** requires collecting 2 ml of venous blood into a tube containing 0.5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4°C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the
lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.

**GBP:**

**CBC:** Done on ABX PENTRA XL80, by Automated Method

**pH:** pH was tested by Litmus paper supplied by Qualigens

**GLUCOSE**

Serum and pleural fluid glucose was estimated by enzymatic, colorimetric, end point, glucose oxidase peroxidase (GOD-POD) method on VITROS 250 automatic analyzer.
**Principle:**
Glucose oxidase oxidizes glucose in the sample and forms hydrogen peroxide and gluconic acid. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a coloured dye. The intensity of the dye is measured by reflected light at 540 nm. The values are reported in mg/dl. The dye system used is closely related to that first reported by Trinder.\(^{33}\) The chemistry of the glucose slides has been described by Curme,\(^{34}\) et al

\[
\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{D-gluconic acid} + H_2O_2
\]

\[
2H_2O_2 + 4\text{-aminoantipyrine} + 1,7\text{-dihydroxynaphthalene} \xrightarrow{\text{Peroxidase}} \text{red dye}
\]

**Reference range:** <200 mg/dl (random)

**Total protein**
Test type is colorimetric, non enzymatic done by Vitros 250 system with TP multilayered slides at 37 degrees Celsius and incubated for 5 minutes.

**Principle:**
The method of analysis is based on the Biuret reaction, in which the reaction takes place between the protein and Copper tartrate to form a violet colored complex at 37 degree centigrade, in the presence of Lithium hydroxide (which provides an alkaline medium). The amount of colored complex formed is proportional to the amount of total protein in the sample and is measured as an end point after 5 min by reflectance spectrophotometry at 540nm.

\[
\text{Protein} + \text{Copper tartrate} \xrightarrow{\text{LiOH}} \text{colored complex}
\]

**Reference range:** 6.7-8.6 g/dl
**LDH:**

**Principle**

Lactate dehydrogenase catalyzes the conversion of pyruvate and NADH to lactate and NAD$^+$. The oxidation of NADH, which is monitored at 340nm by reflectance spectrophotometry at 37 degree for 5 minutes, is used to measure lactate dehydrogenase activity.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightleftharpoons{LDH} \text{Lactate} + \text{NAD}^+$$

**AMYLASE:**

**Principle**

Amylase catalyzes the hydrolysis of dyed starch (dye covalently linked to amylopectin) into smaller dyed saccharides. These dyed saccharides diffuse into the underlying reagent layer.

The reflection density of the dyed saccharide in the reagent layer is measured at 540nm by reflectance spectrophotometry at 37 degree for 5 minutes. The difference in the slide’s reflection density between the two readings is proportional to sample amylase activity.

$$\text{Amylase} \xrightarrow{amylase} \text{Dyed amylopectin \rightarrow dyed saccharides}$$
CKMB:

Principle

Creatine kinase catalyzes the conversion of creatine phosphate and adenosine diphosphate (ADP) to creatine and adenosine triphosphate (ATP). In the presence of glycerol kinase, glycerol is phosphorylated to L-α-glycerophosphate which is then oxidized to dihydroxyacetone phosphate and H₂O₂ in the reaction catalyzed by L-α-glycerophosphate oxidase. Finally, leuco dye is oxidized by hydrogen peroxide in the presence of peroxidase to form a dye.

The low wavelength light cutoff filter on the side support minimizes the blank rate effects of incident light during dye development. Reflection density is measured at 670nm.

The rate of change in reflection density is converted to enzyme activity.

\[
\text{CK-MM + CK-MB} \xrightarrow{\text{anti CK-M anti body}} \text{CK-M inhibition}
\]

\[
\text{Creatine phosphate + ADP} \xrightarrow{\text{NAC,Mg+2 creatine Kinase B}} \text{Creatine + ATP}
\]

\[
\text{Glycerol + ATP} \xrightarrow{\text{glycerol kinase}} \text{L-α-Glycerophosphate + ADP}
\]

\[
\text{L-α-Glycerophosphate + O}_2 \xrightarrow{\text{L-α-glycerophosphate oxidase}} \text{Dihydroxyacetone PO4+ H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Leuco dye} \xrightarrow{\text{peroxidase}} \text{Dye + 2H}_2\text{O}
\]
**Alanine aminotransferase (ALT/SGOT)**

Test type is multiple point rate done by Vitros 250 system with ALT multilayered slide at 37 degree Celsius and incubated for 5 minutes. Wave length used is 340 nm.

**Principle:**

Alanine aminotransferase catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutamate. Lactate dehydrogenase (LDH) then catalyzes the conversion of pyruvate and NADH to lactate and NAD⁺.

The rate of oxidation of NADH is monitored by reflectance spectrophotometry. The rate of change in reflection density is proportional to enzyme activity.

\[
\text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{glutamate}
\]

\[
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^+\]

**Reference range:** 7-41 U/L

---

**Aspartate aminotransferase (AST/SGPT):**

Analysis is based on enzyme coupled oxidation of NADH to NAD⁺. Done by Vitros 250 system with AST slide at 37 degree Celsius and incubated for 5 minutes. Wave length used is 340 nm.

**Principle:**

The amino group of L-aspartate is transferred to alpha keto glutarate in the presence of pyridoxal-5-phosphate (P-5-P) to produce oxaloacetate and L-glutamate. The oxaloacetate formed in the
Deamination of the L-aspartate is converted to malate by malate dehydrogenase (MDH) in the presence of NADH which is oxidized to NAD\(^+\). The rate of oxidation of NADH is monitored by reflectance spectrophotometry at 37 degree Celsius and 340 nm.

\[
\text{L-Aspartate+alpha ketoglutarate} \xrightarrow{\text{AST \& P-5-P}} \text{Oxaloacetate} + \text{L-glutamate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{malate dehydrogenase}} \text{malate} + \text{NAD}^+
\]

**Reference range:** 12-38U/L

**Urea:**

Urea is measured by enzymatic colorimetric assay in serum.

**Principle:**

Urease enzyme acts on urea and ammonia is released which reacts with the indicator to form a dye. The reflection density of the dye is measured at 670 nm and is proportional to the concentration of urea in the sample. The value is given in mg/dl.

\[
\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_2 + \text{CO}_2
\]

\[
\text{NH}_2 + \text{ammonia indicator} \rightarrow \text{dye}
\]

**Reference range:** 15-50mg%

**Creatinine:**

Creatinine measured by two point rate method in serum.

**Principle:**
Creatinine is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product.

Following addition of the sample, the slide is incubated. During the initial reaction phase, endogenous creatine in the sample is oxidized. The resulting change in reflection density is measured at two time points at 670 nm. The difference in reflection density is proportional to the concentration of creatinine present in the sample. The values are given in mg/ml to the concentration of creatinine present in the sample. The values are given in mg/ml

\[
\text{Creatinine} + \text{H}_2\text{O} \rightarrow \text{creatinine} \rightarrow \text{creatine}
\]

\[
\text{Creatine} + \text{H}_2\text{O} \rightarrow \text{creatinine} \rightarrow \text{sarcosine} + \text{urea}
\]

\[
\text{Sarcosine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{glycine} + \text{formaldehyde} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{leuco dye} \rightarrow \text{dye} + \text{2H}_2\text{O}
\]

For urinary Creatinine estimation the spot urine sample was mixed 1 part with 20 parts of reagent grade water and analysed. The results were multiplied by 21 to obtain the Creatinine concentration in original urine sample.
Reference range: 0.6-1.2 mg/dl: male
0.5-1.0 mg/dl: female

**Purified Protein Derivative (PPD)**

The Mantoux test (also known as the Mantoux screening test, Tuberculin Sensitivity Test, Pirquet test, or PPD test for Purified Protein Derivative) is a diagnostic tool for tuberculosis.

A standard dose of 5 Tuberculin Units (0.1 mL) is injected intradermally and read 48 to 72 hours later. A person who has been exposed to the bacteria is expected to mount an immune response in the skin containing the bacterial proteins.

The reaction is read by measuring the diameter of induration (palpable raised hardened area) across the forearm (perpendicular to the long axis) in millimeters.

If there is no induration, the result is recorded as "0 mm".

Erythema (redness) is not measured.

**ADA:**

Kit used from Microxpress subdivision of TULIP Diagnostic (P) LTD. India.

**Reagents used in this study:**

MICROXPRESS ADA-MTB is a reagent for laboratory use only. ADA-MTB comprised of:

1. **L1**- ADA-MTB Reagent
2. **L2**- ADA-MTB Reagent
3. **L3-** ADA-MTB Reagent

4. **L4-** ADA-MTB Reagent

5. **S-** ADA-MTB Reagent

Plate 17: ADA-MTB KIT
Plate 18: Pleural fluid samples (Straw, Turbid, Haemorrhagic)
**Principle**

Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue colored indophenol complex formed is directly proportional to the amount of ADA present in the sample.

\[
\text{ADA} \\
\text{Adenosine} + \text{H}_2\text{O} \rightarrow \text{Ammonia} + \text{Inosine}
\]

\[
\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \rightarrow \text{Blue Indophenol Complex}
\]

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th>Pleural Fluid</th>
<th>Normal</th>
<th>Suspect</th>
<th>Strong</th>
<th>Suspect</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 30 U/L</td>
<td>30 U/L to 40 U/L</td>
<td>&gt; 40 U/L to 60 U/L</td>
<td>&gt; 60 U/L</td>
<td></td>
</tr>
</tbody>
</table>

**STORAGE AND STABILITY**

1. ADA-MTB kit was stored at 2-8°C, away from light.

2. Stability of the ADA-MTB kit was mentioned on the label as per the expiry date.

**NOTE**

1. Kit components from the same lot were used for achieving accurate and reproducible results.
   
   Intermix reagents from same lots were used.

2. Reagents were added sequentially for achieving accurate results.
ADDITIONAL MATERIAL REQUIRED

Test tubes, test tube stand, waterbath/incubator (37°C), distilled or triple distill water, variable volume pipettes, spectrophotometer with filter at 570-630 nm (Hg 578 or 623 nm) at 37°C or colorimeter with yellow or red filter, stopwatch.

REAGENT PREPARATION

Reagents L1, L2 and standard were ready to use. Adenosine Reagent (L2) forms crystals at 2-8°C, so they were dissolved by gently warming the reagent before use. Both the Phenol Reagent (L3) & Hypochlorite Reagent (L4) were diluted 1:5 with distilled water before use (1 part of reagent + 4 parts of distilled water). The Working Phenol Reagent and Working Hypochlorite Reagent were stable for at least 6 months when stored at 2-8°C in tightly closed bottles.

SPECIMEN COLLECTION AND PREPARATION

**Pleuralfluid:**

Site was disinfected and specimen collected with aseptic precautions. ADA is stable in biological fluid for 2 days at 2-8°C as after this, ammonia may be released in the samples even without any microbial contamination.

TEST PROCEDURE

1. All reagents and samples were brought to room temperature before use.

2. Working Phenol and Hypochlorite Reagent were prepared.
3. The spectrophotometer filter was set at 570-630 nm (Hg 578 or 623 nm) at 37°C.

4. Sample was pipetted into clean dry test tubes labeled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows-

<table>
<thead>
<tr>
<th>Addition Sequence</th>
<th>B (ml)</th>
<th>S (ml)</th>
<th>SB (ml)</th>
<th>T (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer Reagent</td>
<td>0.20</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenosine Reagent</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Triple distill water</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard Reagent</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample Reagent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
</tbody>
</table>

5. Mix well and incubate at 37°C for exactly 60 minutes, and then add the following:

| Working Phenol Reagent     | 1.00   | 1.00   | 1.00    | 1.00   |
| Sample Reagent             | -      | -      | 0.02    | -      |
| Working Hypochlorite Reagent | 1.00   | 1.00   | 1.00    | 1.00   |

6. Mix and incubate at 37°C for 15 minutes.

7. Absorbance of the Blank (Abs. B), Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) was measured against distilled water.

**CALCULATIONS**

\[
\text{Total ADA activity in U/L} = \frac{\text{Abs. T - Abs. SB}}{\text{Abs. S - Abs. B}} \times 50
\]

**TB PCR** - Using Bangalore Genei™ amplification kit for mycobacterium tuberculosis