MATERIAL AND METHODS

The present study was conducted in the Department of Biochemistry M.G.M. Medical College and associated M.Y. Hospital, Indore. Patient history was recorded on the proforma and general examination was done for blood pressure, edema and weight gain. The individuals who volunteered for study were grouped as follows:

**Group-A (Control group):** Age matched healthy normotensive non-pregnant women. (N=100 cases).

**Group-B (Control group):** Healthy normotensive pregnant women in third trimester of pregnancy (28-40 weeks) (N=100 cases).

**Group-C (Study group):** Pregnant women with preeclampsia in third trimester of pregnancy (28-40 weeks) (N=100 cases).

This study group was further divided into two subgroups as -

**Subgroup C1** (Mild preeclamptic): Preeclamptic women having BP≥140/90 and proteinuria > 300 mg/24 hours and absence of symptoms. (N=68 cases)

**Subgroup C2** (Severe preeclamptic): Preeclamptic women having BP≥ 160/110, proteinuria >500mg/24 hours and presence of pathological edema and other symptoms. (N=32 cases)

INCLUSION CRITERIA

A - Inclusion criteria for Control Groups A and B:

1) Healthy individuals.

2) Child bearing age between 20-40 years.

B- Inclusion criteria for Study Group C:

1. Child bearing age between 20-40 years.

2. Women with multiple pregnancies (twins/triplets) were included in the study.
EXCLUSION CRITERIA

A - Exclusion criteria for Control Groups A and B:

1. Severe anemic pregnant women were excluded. (Hb <6 gm/dl)
2. Past history of Diabetes, chronic infection, hypertension, chronic renal disease, active urinary tract infection, absence of thromboembolic disease, viral hepatitis.
3. Women using any kind of oral contraceptives or anticoagulant drugs.
4. Smokers, alcoholics and women during labor pains.

B- Exclusion criteria for Study Group C:

1. Patients before 20th week of pregnancy.
2. Past history of Diabetes, chronic infection, hypertension, chronic renal disease, active urinary tract infection, absence of thromboembolic disease, viral or drug induced hepatitis.
3. Women using any kind of oral contraceptives or anticoagulant drugs.
4. Smokers, alcoholics and women during labor pains.
5. Patients showing eclamptic fit.
6. Patients on magnesium sulphate and calcium lactate drugs.
7. Patients with any confounding conditions that altered the coagulation tests such as placental abruption or previa, sepsis, stillborn or heavy vaginal bleeding, premature rupture of membranes were excluded.

COLLECTION OF SAMPLES

Blood samples were collected in citrate/ plain/ EDTA vials for analysis of Coagulation/ Biochemical/Hematological parameters respectively. Sample collection during febrile duration was avoided.

5 ml of venous blood was drawn from the antecubital aseptically and was allowed to clot. Centrifugation of these specimens was done for ten minutes at room temperature and at
2500 x g. Plasma samples for assay of coagulation parameters were stored at -80°C and serum samples were stored at -20°C in deep freezer until analysis. Random urine samples for albumin/creatinine ratio (UACR) were also collected.

Coagulation parameters APTT, PT & Fibrinogen were assayed on **Start analyzer a compact 4-channel coagulation instrument.** FDP was analyzed by **ELISA** method. The AT III was estimated using the fully automatic Coagulometer equipment **Diagnostic Stago STA Compact.** Biochemical parameters were analyzed on **fully automated chemistry analyzer Biosystems A25.** **Hematological parameters** were assayed on **cell counter PCN-201(N).**

The following parameters were analyzed -

1. Plasma APTT
2. Plasma PT
3. Plasma Fibrinogen
4. Plasma FDP
5. Plasma AT III
6. hs-CRP
7. Serum Calcium
8. Serum Magnesium
9. Serum LDH
10. Serum SGPT/ALT
11. Serum SGOT/AST
12. Serum ALP
13. Serum Bilirubin Total
14. Serum Total Protein
15. Serum Albumin  
16. Serum GGT  
17. Serum Urea  
18. Blood Urea Nitrogen (BUN)  
19. Serum Creatinine  
20. Serum Uric Acid  
21. Urine Albumin/ Creatinine Ratio (UACR)  
22. WBC  
23. RBC  
24. Hemoglobin  
25. Hematocrit  
26. Platelets  
27. ESR  

**STATISTICAL ANALYSIS**

Comparison of above said parameters in control and study groups was done by one way analysis of variance (ANNOVA) with post hoc Bonferroni test. Comparison between mild and severe preeclamptic subgroups was done by independent t test. Correlations were calculated by Pearson’s correlation coefficient. The said calculations were made on SPSS software version 20.
ESTIMATION OF PLASMA ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

Estimation of plasma APTT was done by Clotting Assay/Electromagnetic Monitoring of steel ball movement.\(^{246}\)

**Principle**

The APTT involves the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) and a factor XII activator (kaolin). The APTT explores the coagulation factors XII, XI, IX, VIII, X, V, II, and I except the platelets.\(^{246}\)

**Reagent Composition**

**Reagent 1 (R1)**– Cephalin (platelet substitute), prepared from rabbit cerebral tissues according to Bell and Alton\(^{247}\), lyophilized.

**Reagent 2 (R2)** – 5ml vial, buffered suspension of kaolin (5mg kaolin per ml).

**Reagent Preparation**

Shake a vial of Reagent 2 (R2) well and transfer its entire contents into a vial of Reagent 1 (R1). Allow the reconstituted Reagent 1 to stand at room temperature (18-25°C) for 30 minutes. Swirl the Reagent 1 vial gently to obtain a homogenous suspension.

**Reagent Storage**

The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C. Do not freeze.

**Sample Collection, Treatment and Storage**

Sample collections must be in conformity with the recommendations for haemostatis tests.

- 1.8 ml of blood is collected with 0.2 ml of 3.8% of trisodium citrate anticoagulant.
- Centrifugation of blood sample is done for 15 minutes at 2000-2500 g.
- Plasma remains stable for 4 hours at 20 +5 °C.

**Procedure**
1. Place cuvette-strips in the incubation area for prewarming at 37 °C for 3 minutes.

2. Dispense a ball to each cuvette.

<table>
<thead>
<tr>
<th>In prewarmed cuvettes (37 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation Area</strong></td>
</tr>
<tr>
<td>Dispense plasma(control or patient’s)-50 µl</td>
</tr>
<tr>
<td>Pipette Reagent 1 (R1) -50 µl</td>
</tr>
<tr>
<td>Start the timer corresponding to the incubation column for an incubation of 180 sec.</td>
</tr>
<tr>
<td>When the instrument starts to beep, transfer the cuvettes to the test-column area.</td>
</tr>
<tr>
<td>Prime the Finnpipette with the start reagent (SR).</td>
</tr>
<tr>
<td><strong>Test-Column</strong></td>
</tr>
<tr>
<td>Activate the Finnpipette by pressing the pipette key.</td>
</tr>
<tr>
<td>Dispense CaCl₂,0.025 M prewarmed at 37°C - 50 µl</td>
</tr>
</tbody>
</table>

**Reference Range**

Non pregnant women-26.3-39.4 sec
Pregnant women (third trimester)-24.7-35.0 sec
**ESTIMATION OF PLASMA PROTHROMBIN TIME (PT)**

Plasma Prothrombin time estimation was done by Clotting Assay/ Electromagnetic Monitoring of steel ball movement.\(^{249}\)

**Principle**

The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of the patient’s plasma and to compare it with that of a normal standard.\(^{249}\)

The test measures, as a whole, the activity of the coagulation factor:

- Factor II (Prothrombin),
- Factor V (Proaccelerin),
- Factor VII (Proconvertin),
- Factor X (Stuart factor)
- Factor I (Fibrinogen)

**Reagent Composition**

- **Reagent 1 (R1)** – Neoplastine Cl Plus, lyophilized thromboplastin prepared from fresh rabbit cerebral tissues.

- **Reagent 2 (R2)** - Solvent containing calcium, 5 ml per vial or 10 ml per vial.

**Reagent Preparation**

Transfer the entire contents of one vial of Reagent 2 (R2) into one vial of Reagent 1 (R1). Allow the reconstituted reagent to stand at room temperature (18-25°C) for 30 minutes. Swirl the Reagent 1 vial gently to obtain a homogenous suspension.

**Reagent Storage**

The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C. Do not freeze.
Sample Collection, Treatment and Storage

Sample collections must be in conformity with the recommendations for haemostasis tests.

- 1.8 ml of blood is collected with 0.2 ml of 3.8% of trisodium citrate anticoagulant.
- Centrifugation of blood sample is done for 15 minutes at 2000-2500 g.
- Plasma remains stable for 8 hours at 20 ± 5 °C.
- Do not store plasma at 2–8°C.

Procedure

1. Place cuvette-strips in the incubation area for prewarming at 37 °C for 3 minutes.

2. Dispense a ball to each cuvette.

<table>
<thead>
<tr>
<th>In prewarmed cuvettes (37 °C):</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation Area</strong></td>
</tr>
<tr>
<td>Dispense plasma/standard/control-50 µl</td>
</tr>
<tr>
<td>Start the timer corresponding to the incubation column for an incubation of 60 sec.</td>
</tr>
<tr>
<td>When the instrument starts to beep, transfer the cuvettes to the test-column area.</td>
</tr>
<tr>
<td>Prime the Finnpipette with the start reagent (SR).</td>
</tr>
</tbody>
</table>

| **Test-Column**               |
| Activate the Finnpipette by pressing the pipette key. |
| Dispense Reagent 1 prewarmed at 37°C - 100 µl |

**Reference Range**

Non pregnant women-12.7-15.4 sec

Pregnant women (third trimester) - 9.6-12.9 sec
**ESTIMATION OF PLASMA FIBRINOGEN**

Quantitative estimation of plasma Fibrinogen was done by clotting method of Clauss.\textsuperscript{250}

**Principle**

In the presence of an excess of thrombin, the clotting time of diluted plasma has a direct bearing on the level of plasma fibrinogen.\textsuperscript{250}

**Reagent Composition**

Lyophilized titrated human calcium thrombin containing a specific heparin inhibitor to allow the assay of fibrinogen in heparinized plasma samples.

**Reagent Preparation**

Reconstitute each vial of reagent with 2 ml of distilled water. Allow the reconstituted reagent to stand at room temperature (18-25°C) for 30 minutes. Swirl the vial gently.

**Reagent Storage**

The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C. In case of storage at 2-8°C, allow the reagent to stand at room temperature (18-25°C) for 30 minutes before use.

**Sample Collection, Treatment and Storage**

Sample collections must be in conformity with the recommendations for haemostatis tests.

- 1.8 ml of blood is collected with 0.2 ml of 3.8% of trisodium citrate anticoagulant.
- Centrifugation of blood sample is done for 15 minutes at 2000-2500 g.
- Plasma remains stable for 8 hours at 20 +5 °C.
**Procedure**

1. Place cuvette-strips in the incubation area for prewarming at 37 °C for 3 minutes.

2. Dispense a ball to each cuvette.

<table>
<thead>
<tr>
<th>In prewarmed cuvettes (37 °C):</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation Area</strong></td>
</tr>
<tr>
<td>Dispense test sample( plasma/standard/control)-100 µl</td>
</tr>
<tr>
<td>Start the timer corresponding to the incubation column for an incubation of 60 sec.</td>
</tr>
<tr>
<td>When the instrument starts to beep, transfer the cuvettes to the test-column area.</td>
</tr>
<tr>
<td>Prime the Finnpipette with the start reagent (SR).</td>
</tr>
<tr>
<td><strong>Test-Column</strong></td>
</tr>
<tr>
<td>Activate the Finnpipette by pressing the pipette key.</td>
</tr>
<tr>
<td>Dispense reagent - 50 µl</td>
</tr>
</tbody>
</table>

**Reference Range**

- Non pregnant women-200-400 mg/dl
- Pregnant women (third trimester)-373-619 mg/dl

**ESTIMATION OF PLASMA FIBRIN DEGRADATION PRODUCT (FDP)**

Quantitative determination of the plasma fibrin degradation product (FDP) activity level in plasma was done by the ELISA method.\(^{251}\)

**Principle**

The microtiter plate has been pre-coated with a monoclonal antibody specific to FDP. Calibrators or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for FDP. Next, Avidin
conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain FDP, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is determined by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of FDP in the samples is then determined by comparing the O.D. of the samples to the calibration curve.

**Reagent Composition**

1. Pre-coated ready to use 96-well strip plate
2. Calibrator (lyophilized)
3. Calibrator Diluent
4. Detection Reagent A
5. Detection Reagent B
6. Assay Diluent A (2X concentrate)
7. Assay Diluent B (2X concentrate)
8. TMB Substrate
9. Stop Solution
10. Wash Buffer (30X concentrate)
11. Plate sealer for 96 wells

**Reagent Preparation**

Reagent provided is ready to use. All the reagents should be bring to room temperature (18-25°C) before use.

**Reagent Storage**

All the reagents should be kept according to the labels on vials. The Calibrator, Detection Reagent A, Detection Reagent B and the 96 well-strip plate should be stored at -20 °C upon being received. The unused strips should be kept in a sealed bag to
minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown.

**Sample Collection and Storage**

Sample collections must be in conformity with the recommendations for haemostatsis tests.

- 1.8 ml of blood is collected with 0.2 ml of 3.8% of trisodium citrate anticoagulant.
- Centrifuge the plasma sample for 15 minutes at 1,000× g within 30 minutes of collection.
- Do not use haemolysed samples.
- Plasma storage: Samples to be used within 5 days may be stored at 4°C or ≤ 1 month at -20 °C or ≤2 months at -80 °C to avoid loss of bioactivity and contamination.
- When performing assay slowly bring samples to room temperature.

**Sample Preparation**

Plasma samples require a 1000 fold dilution as per the requirement.

**Calibrator Preparation**

Calibrator is reconstituted with 1.0mL of Calibrator Diluent. The concentration of the calibrator in the stock solution is 10ng/ml. It is diluted to obtain the strength of 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312 ng/mL, 0.156 ng/mL.

**Procedure**

1. Prepare all reagents, samples and calibrators.
2. Add 100 µL calibrator or sample to each well. Incubate 2 hours at 37°C.
3. Add 100 µL prepared Detection Reagent A. Incubate 1 hour at 37°C.
4. Aspirate and wash 3 times.
5. Add 100 µL prepared Detection Reagent B. Incubate 30 minutes at 37°C.
6. Aspirate and wash 5 times.
7. Add 90 µL Substrate Solution. Incubate 10-15 minutes at 37°C.
8. Add 50 µL Stop Solution. Read at 450 nm immediately.

**Calculation of Results**

A calibration curve is created on log-log graph paper. The calibration curve concentrations used for the ELISA’s were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, 0.156 ng/mL.

**Reference Range**

0.156-10 ng/mL

**CONVERSION OF ng/mL to µg/mL**

The value of FDP coming in ng/mL is divided by 1000 and multiplied by dilution factor (1000). The value of FDP then comes in µg/mL.

**ESTIMATION OF PLASMA ANTITHROMBIN III (AT III)**

Quantitative determination of the plasma Antithrombin III (AT III) activity level in plasma was done by the synthetic Chromogenic Substrate method.

**Principle**

Antithrombin exerts a powerful and immediate inhibitory action on thrombin when heparin is present. AT III test procedure consists of two steps:

- **first**, the plasma to be tested is incubated with a known excess of thrombin (Reagent 1) in the presence of heparin.

- The residual thrombin is quantitated by its amidolytic action on the synthetic chromogenic substrate (Reagent 2). (pNA release measured at 405 nm). Since the quantity of thrombin that is neutralized in the first reaction step is proportional to the level present in the plasma being tested, it follows that the residual thrombin in the second reaction step (as measured by the pNA release) is inversely proportional to the AT level of the tested plasma.

**Reagent Composition**
Reagent 1—Approximately 11.3 nkat of bovine thrombin per ml after reconstituted lyophilized.

Reagent 2 – Chromogenic substrate, approx. 1.4µmol per ml after reconstituted, lyophilized.

Reagent 3- 3-ml vial or 6-ml vial of solvent containing heparin.

Reagent Preparation

The reagents in intact vials are stable until the expiry date indicated on the box label, when stored at 2-8°C.

Reagent 1- Take one vial of Reagent 3 (R3) and shake it well. Then, pour its entire contents into a vial of Reagent 1. Allow the reconstituted reagent to stand at room temperature (18-25 °C) for 60 minutes. Swirl vial gently.

Reagent 2 - Reconstitute each vial with 3 ml or 6 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Swirl vial gently. Do not freeze.

Reagent 3- Reagent (R3) is ready to use. Shake vial well before use to reconstitute Reagent 1.

Reagent Storage

The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C. In case of storage at 2-8°C, allow the reagent to stand at room temperature (18-25°C) for 30 minutes before use.

Sample Collection, Treatment and Storage

Sample collections must be in conformity with the recommendations for haemostatsis tests.

- 1.8 ml of blood is collected with 0.2 ml of 3.8% of trisodium citrate anticoagulant.
- Centrifugation of blood sample is done for 15 minutes at 2000-2500 g.
- Plasma remains stable for 8 hours at 20 +5 °C.
- 1 month at -20 °C. Place the sample at 37 °C, the necessary and sufficient time to obtain the complete thawing.

**Procedure**

<table>
<thead>
<tr>
<th>Citrated plasma</th>
<th>1 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin Reagent</td>
<td>60 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 3 minutes at 37 ºC

| Substrate Reagent | 10 µl |

Determine \( A_{405nm} \) /minute

**Reference Range**

Non pregnant women-80-120%

Pregnant women (third trimester)-82-116%

**ESTIMATION OF SERUM hs C-REACTIVE PROTEIN (hs-CRP)**

Serum hs-CRP was estimated by Immunoturbidimetric latex- high sensitivity method.

**Principle**

Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

**Reagents Composition**

- Reagent A-Glycine buffer (0.1 mol/L),sodium azide (0.95 gm/l),pH 8.6
- Reagent B-Suspension of latex particles coated with anti human C-reactive protein antibodies, sodium azide (0.95gm/L)
- Reagent S-hs- CRP Standard

**Reagent Preparation**
**Working Reagent**: Pour the contents of a Reagent B vial into a Reagent A bottle. Mix thoroughly. Stable for 20 days at 2-8°C.

Smaller Working Reagents volumes can be prepared by mixing 1 mL of Reagent B + 9 mL of Reagent A. Shake the Reagent B vial before pipetting.

**hs-CRP Standard (S)**-Reconstitute with 5.0 mL of distilled water. Stable for 1 month at 2-8 °C.

**Calibration curve**: Prepare dilution of the hs-CRP Standard using 9g/L saline as diluents. Multiply the concentration of the hs-CRP Standard by the corresponding factor indicated below to obtain the hs-CRP concentration of the dilution.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (µL)</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Saline (µL)</td>
<td>70</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

| Factor | 0.125 | 0.25  | 0.5   | 0.75  | 1.0  |

**Reagent Storage**

Store at 2-8°C. Reagents and Standards are stable until the expiry date shown on the label when stored tightly and if contaminations are prevented during their use.

**Sample**

Serum is stable for 7 days at 2-8 °C.

**Procedure**

1. Bring the Working Reagent to 37 °C.

2. Zero the instrument with distilled water.

3. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>Sample/standard/ water(blank)</th>
<th>20 µL</th>
</tr>
</thead>
</table>
Mix and immediately insert the cuvette into the instrument. Start stopwatch.

Record the absorbance at 540 nm and after 10 seconds (A₁) and after 5 seconds (A₂).

Calculations

**Calibration curve:** Calculate the absorbance difference (A\text{Standard} - A\text{Blank}) of each point of the calibration curve and plot the values found against the hs-CRP concentration. hs-CRP concentration in the sample is calculated by interpolation of its absorbance (A\text{Standard} - A\text{Blank}) on the calibration curve.

**Reference Range**

<3.33 mg/L

**ESTIMATION OF SERUM MAGNESIUM**

Serum magnesium was estimated by Spectrophotometric Calmagite method.²⁵⁴

**Principle**

Magnesium in the sample reacts with Calmagite in alkaline medium forming a colored complex that can be measured by spectrophotometry. EGTA is included in the reagent to remove calcium interference.²⁵⁴

\[
\text{Magnesium} + \text{Calmagite (Sample)} \rightarrow \text{Alkaline medium} \rightarrow \text{colored complex}
\]

**Reagent Composition**

A-Calmagite 80 mmol/L, EGTA 60 mmol/L, diethyl amine 0.2 mol/L

B-Magnesium standard -2 mg/dL

**Reagent Preparation**
Reagent provided is ready to use.

Reagent Storage

Store at 2-8°C. Reagents and Standards are stable until the expiry date shown on the label when stored tightly and if contamination is prevented during their use.

Sample

Serum sample should be unhaemolysed. Magnesium in serum is stable for 10 days at 2-8°C.

Procedure

1. Bring the Reagent to room temperature.
2. Pipette into labeled test tube:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Standard</td>
<td>-----</td>
<td>10 µL</td>
<td>-----</td>
</tr>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
<td>10 µL</td>
</tr>
<tr>
<td>Reagent (A)</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

2 Mix thoroughly and let stand the tubes for 2 minutes at room temperature.

3 Read the absorbance (A) of the Standard and the Sample at 520 nm against the Blank.
   The color is stable for at least for 1 hour.

Calculation

The magnesium concentration in the sample is calculated using the following formula:

\[
\text{Serum magnesium (mg/dL)} = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} \times \text{conc. of standard (mg/dL)}
\]

Linearity

Linearity limit is 4 mg/dL
Reference Range

1.7-2.4mg/dL

**ESTIMATION OF SERUM LACTATE DEHYDROGENASE (LDH)**

Serum Lactate dehydrogenase estimation was done by Continuous- spectrophotometric PYRUVATE method.²⁵⁵

**Principle**

Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate by NADH to form lactate and NAD⁺. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm.²⁵⁵

\[
\text{LDH} \quad \text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + \text{NAD}^+
\]

**Reagent Composition**

**Reagent A:** Tris 80mmol/L, pyruvate 1.6 mmol/L, sodium chloride 200 mmol/L. pH 7.2.

**Reagent B:** NADH 3.1 mmol/L, sodium azide 9.5g/L.

**Reagent Preparation**
**Working Reagent**—Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 9mL Reagent A+1mL Reagent B. Stable for 2 months at 2-8 °C.

**Reagent Storage**

Store at 2-8 °C. Reagents are stable until the expiry date shown on the label when stored tightly and if contamination is prevented during their use.

**Sample**

Unhaemolysed serum sample. Lactate dehydrogenase in serum is stable for 24 hours at 2-8 °C and for 2 days at room temperature.

**Procedure**

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>1.0 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 30 seconds, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbance and the average Absorbance difference per minute (ΔA/min).

**Calculation**

The LDH concentration in the sample is calculated using the following general formula:

\[ \Delta A/\text{min} \times \frac{V_t \times 10^6}{\epsilon \times 1 \times} = \text{U/L} \]
The molar absorbance (ε) of NADH at 340 nm is 6300 and the light path (l) is 1 cm, the total reaction volume (Vt) is 1.02 the sample volume (Vs) is 0.02 and 1 U/L are 0.0166µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

\[ \Delta A/\text{min} \times 8095 = \text{U/L} \]

**Linearity**

Linearity limit is 1250 U/L

**Reference Range**

204-414 U/L at 37 °C

**ESTIMATION OF SERUM GAMMA-GLUTAMYL TRANSFERASE (γ-GT)**

Serum Gamma glutamyl transferase was estimated by Continuous- spectrophotometric IFCC method.²⁵⁶

**Principle**

Gamma-glutamyltransferase (GGT) catalyzes the transfer of the γ-glutamyl group from γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine, liberating 3-carboxy-4-nitroaniline. The catalytic concentration is determined from the rate of 3-carboxy-4-nitroaniline formation.²⁵⁶

\[ \text{GGT} \]
\[ \gamma\text{-Glutamyl-3-carboxy-4-nitroanilide} + \text{Glycylglycine} \rightarrow \gamma\text{-Glutamyl-glycylglycine} + 3\text{-carboxy-4-nitroaniline} \]

**Sample**
Serum sample should be unhaemolysed. Gamma-glutamyltransferase in serum is stable for 5 days at 2-8°C.

**Procedure**

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette:
   
<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>1.0 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

3. Mix and insert the cuvette into the photometer.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbance and the average absorbance difference per minute (ΔA/min).

**Calculation**

The GGT concentration in the sample is calculated using the following general formula:

\[ \frac{V_t \times 10^6}{\Delta A/\text{min} \times \epsilon \times 1 \times V_s} = \text{U/L} \]

The molar absorbance (ε) of 3-carboxy-4-nitroaniline at 410 nm is 7908 and at 405 nm is 9900, the light path (l) is 1 cm, the total reaction volume (Vt) is 1.1 the sample volume (Vs) is 0.1 and 1 U/L are 16.67 nkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

<table>
<thead>
<tr>
<th>405 nm</th>
<th>410nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔA/minute × 1111 = U/L</td>
<td>ΔA/minute × 1391 = U/L</td>
</tr>
</tbody>
</table>

**LINEARITY** - Linearity limit is 300 U/L
Reference Range

12-48 U/L.

CALCULATION OF MICROALBUMIN

\[
\text{Micro albumin (mg/L)} = \frac{\Delta A \text{ of Sample}}{\Delta A \text{ of Standard}} \times \text{conc. of standard (mg/L)}
\]

Micro albumin (mg/day) = Micro albumin (mg/L) × Urine Vol. in ml (24hrs)

Reference Range

Normal adults: upto20 mg/L

CALCULATION OF URINE ALBUMIN/CREATININE RATIO (UACR)

\[
\text{Urine creatinin (mg/dl)} = \frac{\Delta A \text{ of Sample}}{\Delta A \text{ of Standard}} \times \text{conc. of standard (mg/dl)} \times 20
\]

\[
\text{UACR in mg/Gm} = \frac{\text{urine albumin (mg/dL)}}{\text{urine creatinin (gm/dL)}} = \text{Albumin excretion mg/day}
\]

Reference Range

UACR: < 30 mg/gm

Compilation of the parameters studied along with their methods and reference range

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Method</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plasma APTT</td>
<td>Clotting Assay / Electromagnetic Monitoring of steel ball movement.</td>
<td>26.3-39.4 sec</td>
</tr>
<tr>
<td>2</td>
<td>Plasma PT</td>
<td>Clotting Assay / Electromagnetic Monitoring of steel ball movement.</td>
<td>12.7-15.4 sec</td>
</tr>
<tr>
<td>3</td>
<td>Plasma Fibrinogen</td>
<td>Clotting method of Clauss.</td>
<td>200-400 mg/dl</td>
</tr>
<tr>
<td>4</td>
<td>Plasma FDP</td>
<td>ELISA</td>
<td>0.156-10 ng/mL</td>
</tr>
<tr>
<td>5</td>
<td>Plasma Antithrombin-III</td>
<td>Synthetic chromogenic Substrate</td>
<td>80-120%</td>
</tr>
<tr>
<td>6</td>
<td>Serum hs-CRP</td>
<td>Latex-High sensitivity (Turbidimetry)</td>
<td>&lt;3.33 mg/L</td>
</tr>
<tr>
<td>7</td>
<td>Serum Calcium</td>
<td>Arsenazo III</td>
<td>8.6-10.3 mg/dL</td>
</tr>
<tr>
<td>8</td>
<td>Serum Magnesium</td>
<td>Calmagite</td>
<td>1.7-2.4 mg/dL</td>
</tr>
<tr>
<td>9</td>
<td>Serum LDH</td>
<td>Pyruvate</td>
<td>207-414 U/L</td>
</tr>
<tr>
<td>10</td>
<td>Serum ALT/SGPT</td>
<td>IFCC kinetic</td>
<td>5-41 U/L</td>
</tr>
<tr>
<td>11</td>
<td>Serum AST/SGOT</td>
<td>IFCC kinetic</td>
<td>5-40 U/L</td>
</tr>
<tr>
<td>12</td>
<td>Serum ALP</td>
<td>IFCC AMP buffer</td>
<td>Upto 105 U/L</td>
</tr>
<tr>
<td>13</td>
<td>Serum Bilirubin total</td>
<td>Diazotized Sulfanilic</td>
<td>0.2-1.0 mg/dL</td>
</tr>
<tr>
<td>14</td>
<td>Serum Total protein</td>
<td>Biuret</td>
<td>60-80g/L or 6.0-</td>
</tr>
<tr>
<td>No.</td>
<td>Test Description</td>
<td>Method/Lab</td>
<td>Reference Range</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>15</td>
<td>Serum Albumin</td>
<td>Bromocresol green (BCG)</td>
<td>35-50g/L or 3.5-5.0g/dl</td>
</tr>
<tr>
<td>16</td>
<td>Serum GGT</td>
<td>Continuous spectrophotometric IFCC</td>
<td>12-48U/L</td>
</tr>
<tr>
<td>17</td>
<td>Serum Urea/BUN</td>
<td>Urease/Glutamate dehydrogenase</td>
<td>Urea-15-39mg/dL BUN-7-18mg/dL</td>
</tr>
<tr>
<td>18</td>
<td>Serum Creatinine</td>
<td>Alkaline picrate</td>
<td>0.6-1.1 mg/dL</td>
</tr>
<tr>
<td>19</td>
<td>Serum Uric Acid</td>
<td>Uricase/peroxidase</td>
<td>2.6-6.0mg/dL</td>
</tr>
<tr>
<td>20</td>
<td>Urine microalbumin</td>
<td>Latex immunoturbidimetric</td>
<td>Upto20 mg/L</td>
</tr>
<tr>
<td>21</td>
<td>Urine Creatinine</td>
<td>Alkaline picrate</td>
<td>Urine Creatinine- 11-26 mg/kg/day</td>
</tr>
<tr>
<td>22</td>
<td>Urine albumin / creatinine ratio (UACR)</td>
<td>Calculated</td>
<td>UACR: &lt; 30 mg/gm</td>
</tr>
<tr>
<td>23</td>
<td>WBC</td>
<td>Cell Counter [PCE-201(N)]</td>
<td>4,000-11,000/cumm</td>
</tr>
<tr>
<td>24</td>
<td>RBC</td>
<td>Cell Counter [PCE-201(N)]</td>
<td>4.2-5.4 millions/cumm</td>
</tr>
<tr>
<td>25</td>
<td>Hemoglobin</td>
<td>Cell Counter (Cyanmethemoglobin) [PCE-201(N)]</td>
<td>12-16 g/dl</td>
</tr>
<tr>
<td>26</td>
<td>Hematocrit</td>
<td>Cell Counter [PCE-201(N)]</td>
<td>37%-47%</td>
</tr>
<tr>
<td>27</td>
<td>Platelet counts</td>
<td>Cell Counter [PCE-201(N)]</td>
<td>1.65-4.15 lakhs/cumm</td>
</tr>
<tr>
<td>28</td>
<td>ESR</td>
<td>Wintrobe’s</td>
<td>0-20 mmHg</td>
</tr>
</tbody>
</table>