Appendix
PREPARATION OF REAGENTS / SOLUTIONS

1. For acetylcholinesterase assay:

a) **Stock buffer**

Sodium barbital ......... 12.37 g
Potassium dihydrogen phosphate ......... 1.36 g
Sodium chloride ......... 175.35 g

The above chemicals were dissolved in 1 l distilled water.

b) **Stock Buffer Indicator** (Stock BI)

100 mg of bromothymol blue was dissolved in 2 ml of 2M sodium hydroxide in 1 l volumetric flask. To this, 150 ml of the stock buffer solution was added and then diluted to approximately 950 ml with distilled water. The pH was adjusted to 8.0 by adding about 16 ml of 0.5 N HCl.

c) **Working BI solution**

476.2 ml of stock BI solution was diluted to 1 l with distilled water.
2. For histopathological techniques :

a) **Buffered neutral formalin solution**
   To about 900 ml of distilled water, 4 g of sodium phosphate monobasic, 6.5 g of sodium phosphate dibasic and 100 ml of 40 per cent formalin was added.

b) **Harris haematoxylin**
   5 g of haematoxylin was dissolved in 50 ml of absolute alcohol and 100 g potassium alum in 1 l of distilled water by aid of heat. After removing from heat both the solutions were mixed, boiled and 2.5 g of mercuric oxide was added after removing from heat. Again the solution was heated, cooled and to 100 ml of this solution 2 to 4 ml of glacial acetic acid was added which increases the precision of nuclear stain. It was filtered before use.

c) **Stock alcoholic eosin solution**
   1 g eosin Y (water soluble) was dissolved in 20 ml of distilled water and 80 ml of 95 per cent alcohol was added to it.

d) **Working eosin solution**
   To 1 part of stock alcoholic eosin solution, 3 parts of 80 per cent alcohol was added. To 100 ml of the stain 0.5 ml of glacial acetic acid was added before use and stirred.
e) **Orange G 6 (OG6) solution**

15 mg of phosphotungstic acid was dissolved in 0.5 per cent of orange G solution made in 95 per cent ethyl alcohol.

f) **Eosin - Azure 50 (EA 50)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 % light green SF</td>
<td>45 ml</td>
</tr>
<tr>
<td>0.5 % eosin WS yellow</td>
<td>45 ml</td>
</tr>
<tr>
<td>0.5 % Bismark brown</td>
<td>45 ml</td>
</tr>
<tr>
<td>Phosphotungstic acid</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Saturated aqueous lithium carbonate</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

g) **May - Grunwald - Giemsa technique**

i) **Stock solution**

0.3 g of powdered May-Grunwald dye was grinded in little methanol and final volume was made up to 100 ml.

ii) **Working May - Grunwald solution**

20 parts of May-Grunwald solution were diluted with 30 parts of phosphate buffer (pH 6.0).

iii) **Phosphate buffer**

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/15 sodium phosphate (cibasic)</td>
<td>49.6 ml</td>
</tr>
<tr>
<td>(9.465 g Na₂HPO₄ / 1 of dis. water)</td>
<td></td>
</tr>
<tr>
<td>M/15 potassium phosphate (monobasic)</td>
<td>50.4 ml</td>
</tr>
<tr>
<td>(9.07 g KH₂PO₄ / 1 of dis. water)</td>
<td></td>
</tr>
</tbody>
</table>
iv) Giemsa stock solution
Availed readily from a commercial firm.

v) Giemsa working solution
10 parts of Giemsa was mixed in 40 parts of phosphate buffer (pH 6.8).

3. For quantitative estimation of DNA:
   a) Dische diphenylamine (DPA) reagent
      1 g of diphenylamine was dissolved in 1 l of glacial acetic acid and 25 ml of concentrated sulphuric acid was added afterwards. The solution was prepared fresh.
   b) Standard DNA solution
      10 mg of DNA (crystals commercially available) was dissolved in 1 dl of distilled water with a magnetic stirrer.

4. For quantitative estimation of RNA:
   a) Orcinol reagent
      100 mg of ferric chloride was dissolved in 100 ml of concentrated HCl and 1 g of orcinol was added to it. The solution was prepared fresh.
   b) Standard RNA solution
      10 mg of RNA (crystals) was dissolved by magnetic stirrer in 1 dl of distilled water.
5. For quantitative estimation of proteins:

a) Folin-Ciocalteau reagent
   Folin-Ciocalteau (commercial) reagent was diluted to 1:1 (v/v) with distilled water.

b) 1% copper sulphate solution
   1 g of CuSO₄ was dissolved in 100 ml of distilled water.

c) 2% sodium carbonate solution
   Prepared in 0.1 N NaOH solution

d) Sodium-Potassium tartarate (2%) prepare in distilled water

5. Reagents for phosphatases (ALP and ACP)

a) Carbonate-bicarbonate buffer (pH 10.0)
   6.3 g of anhydrous sodium carbonate (Na₂CO₃) and 3.36 g of sodium bicarbonate (NaHCO₃) were dissolved in 1 l of distilled water. The pH was adjusted to 10.0 and the buffer was stored at 4°C using a few drops of chloroform as preservative.
b) **Citrate buffer (pH 4.9)**

42 g of citrate (citric acid) was dissolved in little distilled water; to it 376 ml of 1 M sodium hydroxide was added and the volume was made to 1 l with distilled water. The pH was adjusted and the buffer was stored at 4°C using a few drops of chloroform.

c) **Substrate**

1.09 g of disodium phenyl phosphate was added in 500 ml of distilled water. The solution was quickly boiled and cooled. Chloroform was added as a preservative.

d) **Stock phenol standard solution**

1 g phenol was dissolved in 1 l of 0.1 M HCl and the solution was kept in a dark bottle.

e) **Phenol working standard solution**

Stock phenol standard solution was diluted 1 : 100 (v/v) with distilled water.

f) **0.5 M sodium hydroxide solution**

2 g of NaOH was dissolved in 100 ml of distilled water.

g) **0.5 M sodium bicarbonate solution**

4.2 g NaHCO₃ was dissolved in 100 ml of distilled water.

h) **Amino-antipyrine solution**

6 g of 4-aminoantipyrine was dissolved in 1 l of distilled water and stored in dark bottle.
1) **Potassium ferricyanide solution**

24 g of potassium ferricyanide was dissolved in 1 l of water and stored in dark bottle.

7. **Reagents for transaminases** :

a) **Phosphate buffer** (pH 7.4)

\[
\begin{align*}
0.1 \text{ M disodium hydrogen phosphate} & \quad \text{...} \quad 8.4 \text{ ml} \\
(17.8 \text{ g of } \text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O} / 1 \text{ dis. H}_2\text{O})
\end{align*}
\]

\[
\begin{align*}
0.1 \text{ M potassium dihydrogen phosphate} & \quad \text{...} \quad 160 \text{ ml} \\
(13.6 \text{ g } \text{KH}_2\text{PO}_4 / 1 \text{ of dis. H}_2\text{O})
\end{align*}
\]

Few drops of chloroform were added and stored at 4°C.

b) **Substrate for AST**

13.3 g of aspartic acid was dissolved in 90 ml of distilled water and pH adjusted to 7.4. 0.146 g of - ketoglutaric acid was added and again pH adjusted to 7.4 and volume was made upto 500 ml with buffer.

c) **Substrate for ALT**

9.0 g of alanine was dissolved in 90 ml of distilled water and 2.5 ml of N NaOH solution was added to adjust pH to 7.4. Further, 0.146 g of - ketoglutaric acid was dissolved in it and pH adjusted to 7.4; the volume was made upto 500 ml with phosphate buffer.
d) **Stock pyruvate standard**

220 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer and stored at 4°C.

e) **Working pyruvate standard solution**

Stock pyruvate solution diluted 5 times with phosphate buffer.

f) **DNPH solution**

19.8 mg of 2, 4-dinitrophenylhydrazine was dissolved in 10 ml of concentrated HCl and made to 100 ml with distilled water.

g) **0.4 M NaOH solution**

16 g of NaOH was dissolved in 1 l of distilled water.

h) **For Cytochrome P-450**

a) **0.2 M potassium phosphate buffer, pH 7.5**

To 9.5 ml of 0.2 M potassium dihydrogen phosphate, 40.5 ml of 0.2 M dipotassium hydrogen phosphate was added and pH adjusted to 7.5.

b) **0.3 M sucrose solution**

Prepared in 0.2 M phosphate buffer.

c) **0.1 M potassium phosphate buffer pH, 7.5**

To 8.40 ml of 0.1 M dipotassium hydrogen phosphate, 160 ml of 0.1 M potassium dihydrogen phosphate was added and pH adjusted to 7.5.
9. For Alizarin red - S staining technique:

Alizarin red - S

1 mg of Alizarin red-S was dissolved in 1 dl of 1% KOH.