Appendix
I) Reagents and stains

a) Griess Illowskey reagent

Solln I
Sulphanillic acid 0.8g
Acetic acid 1000ml

Solln II
N,N dimethyl 1-1 naphthylamine 0.6g
Acetic acid 100ml

b) Oxidase reagent

Tetramethyl p- phenylenediamine : 1.0g
Isoamyl alcohol : 1.0g

c) Folin Lowrys reagents

Reagent A : 2g Na<sub>2</sub>CO<sub>3</sub> + 0.4 g NaOH in 100ml D/W
Reagent B : 0.5% CuSO<sub>4</sub> solution in 1% Sodium pottasium tartarate
Reagent C : 50ml of A + 1ml of B

Folin Ciocalteaux reagent : Dilute 1:3

d) Kovacs reagent

Para dimethyl amino bezalydehyde : 5.0g
Isoamyl alcohol : 75ml
HCl (37%) :25ml

e) Erlich Reagent

p-dimethyl amino benzalydehyde : 4.0g
absolute alcohol : 380ml
conc HCL : 80ml
f) **Grams stains**

Stain the desalted and fixed smear with Crystal violet for 30 sec. Pour off and treat with Gram's iodine for 1 min. Wash with water. Decolorise with 70% ethanol. Wash with water. Counter stain with saffranine for 40 sec. Wash with water. Air dry and mount under oil immersion lens.

i) **Crystal violet**

Dissolve 20 g of crystal violet in 200 ml of methylated (rectified) spirit. Dissolve 8 g of ammonium oxalate in 800 ml of D/W mix both together to make 100ml

ii) **Gram’s iodine**

Iodine : 10g
KI : 20g
Mix well and dissolve in 100 ml D/W

iii) **70% alcohol**

absolute alcohol : 700ml
D/W : 300ml

iv) **Saffranine (0.5%)**

saffranine : 500mg
D/W : 100ml

Grind the stain with pestle and motor with 10ml and make to 100ml.

g) **Arsenic Estimation Reagents**

i) **Standard iodine solution (0.05N)**

Iodine : 6.5g
Potassium iodate : 20g
Adjust volume to 500 ml with D/W
Store away from heat and light
ii) **Methyl red solution**

Methyl red : 0.05g  
D/W : 100ml

iii) **Starch indicator**

Starch : 1g  
D/W : 100ml

iv) **Sodium thiosulfate**

Na$_2$S$_2$O$_3$.5H$_2$O : 12.5g  
NaCO$_3$ : 0.1g  
Dissolve in 500ml of boiled D/w  
Store in well stoppered bottle

h) **Cadmium estimation reagents**

i) **Dithizone Reagent A**

Dithizone : 50mg  
Chloroform : 500ml  
(to be prepared freshly before use)

ii) **Hydroxyl ammonium chloride solution**

Hydroxyl ammonium chloride : 10g  
Double distilled water : 34ml

iii) **Sodium hydroxide potassium cyanide solution**

NaOH : 40 g  
KCN : 1g  
Double distilled water : 80 ml
iv) Cadion 2B Reagent

4- Nitronapthalene - diazoamino -azo- benzene : 0.02g
ethanol : 100 ml
2M KOH : 1ml
(to be prepared freshly before use)

II) 1 Molar stock solution of Metal salts

a) CdCl$_2$

CdCl$_2$ : 1.834g
Sterile D/W : 10ml

b) Cd(NO$_3$)$_2$ .4H$_2$O

Cd(NO$_3$)$_2$ .4H$_2$O : 3.085g
Sterile D/W : 10 ml

c) Na$_2$HAsO$_4$ .7H$_2$O

Na$_2$HAsO$_4$ .7H$_2$O : 3.119 g
Sterile D/W : 10 ml

d) NaAsO$_2$

NaAsO$_2$ : 1.296 g
Sterile D/W : 10 ml
Reagents for Polyacrylamide Gel electrophoresis (SDS – PAGE)

a) Upper Tris (0.1N)
Tris base : 3.03 g
SDS : 0.2 g
D/W : 40 ml
- pH adjusted to 6.8 with conc. HCl
- Final volume to 50 ml
- Keep at 0-4°C

b) Lower Tris (1.5M)
Tris base : 18.15 g
SDS : 0.4 g
D/W : 90 ml
- pH adjusted to 8.8 (conc. HCl)
- Final volume to 100 ml

c) Acrylamide
Acrylamide : 29.2 g
Bisacrylamide : 0.8 g
D/W : 100 ml
Filter through whatman filter paper

d) Sample buffer (Laemmli, 1970)
Upper tris (pH 6.8) : 1.25 ml
10% SDS : 3.0 ml
glycerol : 1.0 ml
mercaptoethanol : 0.50 ml
D/W : 4.75ml

Freeze

e) Running buffer
Tris base : 3.03g
SDS : 1.0g
Glycine : 14.4g
pH to 8.3
Final volume 1L

f) Staining solution
Coomasie brilliant blue : 1.0g
methanol : 500ml
Acetic acid : 100ml
D/W : 40ml
Filter

g) Destaining soln I / II
Methanol : 500ml / 70ml
Acetic acid : 100ml / 50ml
D/W : 1L / 1 L

h) Tracking dye
Bromophenol blue : 0.1g
50% sucrose : 100ml

i) Fixative for coomasie
Methanol : 50 ml
Glacial acetic acid : 5 ml
Distilled water : 45 ml
j) **Fixative for PAS**

- Ethanol 40 ml
- Glacial acetic acid 5 ml
- D/W 55 ml

Store at R.T.

k) **Periodic acid solution (07 %)**

- Periodic acid 1.4 g
- 5 % acetic acid 200 ml

l) **Sodium metabisulfite (0.2 %)**

- Sodium metabisulfite 0.4 g
- 5 % acetic acid 200 ml
## Composition for preparation of gel

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Constituents</th>
<th>Separating gel</th>
<th>Stacking Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10% T 0.27% C</td>
<td>4% T 0.21% C</td>
</tr>
<tr>
<td>1</td>
<td>30% T 8% C</td>
<td>8.4 ml</td>
<td>1.6 ml</td>
</tr>
<tr>
<td>2</td>
<td>Lower Tris</td>
<td>6.3 ml</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Upper Tris</td>
<td>-</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>4</td>
<td>D/W</td>
<td>10.4 ml</td>
<td>4.8 ml</td>
</tr>
<tr>
<td>5</td>
<td>APS (10%)</td>
<td>250 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>6</td>
<td>TEMED</td>
<td>20 µl</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

- **APS** — Ammonium per sulphate
- **T** — Acrylamide concentration expressed in terms of % T
- **C** — Bis acrylamide concentration expressed in terms of % C
- **TEMED** — Tetra ethylene methyl ethylene diamine
IV) Reagents for plasmid isolation

a) Solution I

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>50 mM</td>
</tr>
<tr>
<td>Tris Cl₂ (pH 8)</td>
<td>25 mM</td>
</tr>
<tr>
<td>EDTA (pH 8)</td>
<td>10 mM</td>
</tr>
</tbody>
</table>

Solution I to be prepared in batches of 100 ml, autoclaved for 15 min at 10 lbs / sq inch and stored at 4°C.

b) Solution II

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 N NaOH</td>
<td>(freshly diluted from 10N stock)</td>
</tr>
<tr>
<td>SDS</td>
<td>1%</td>
</tr>
</tbody>
</table>

c) Solution III

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M potassium acetate</td>
<td>60 ml</td>
</tr>
<tr>
<td>glacial acetic acid</td>
<td>11.5 ml</td>
</tr>
<tr>
<td>D/W</td>
<td>28.5 ml</td>
</tr>
</tbody>
</table>

Resulting solution is 3 M w.r.t potassium acetate and 5 M acetic acid.

d) DNA loading dye

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>2.7 ml</td>
</tr>
<tr>
<td>(5X) TAE</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>SDS (1%)</td>
<td>1 ml</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5M</td>
</tr>
<tr>
<td>Bromothymol</td>
<td>0.25%</td>
</tr>
<tr>
<td>Blue</td>
<td></td>
</tr>
</tbody>
</table>

e) TE Buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mM Tris chloride (pH 8)</td>
<td></td>
</tr>
<tr>
<td>1mM EDTA (pH 8)</td>
<td></td>
</tr>
</tbody>
</table>
f) 40X TAE buffer

Tris base (1.6 M) : 193.6 g
Na acetate .3HO₂ (0.8 M) : 108.9 g
EDTA-Na₂ . 2H₂O (40 mM) : 15.2 g
- pH to 7.2 with CH₃COOH
- water to make to 1 L

g) Agarose gel

Agarose : 0.8g
TAE (IX) : 100 ml
Ethidium Bromide : 4-5 ml
(10mg / ml stock)

h) Lysing solution

SDS : 3%
50 mM Tris pH 12.6
adjust volume to 100 ml with D/W

V) Inhibitors

a) DCCD (1 M Stock)
DCCD : 1.031g
methanol : 5 ml
5 µl of stock added to 5ml cell suspension to obtain a concentration of 1mM

b) p-chloromercuribenzoate (1M stock)
p-chloromercuribenzoate : 0.7g
Distilled water : 2 ml
c) Sodium azide (1M stock)
Sodium azide : 0.13 g
Distilled water : 2 ml.

V) Preparation of Thin layer chromatography plates

Thin layer silica gel plates were prepared by dissolving silica gel G / H / 60F250, (Acme / Qualigens / Merck, India) in distilled water to obtain slurry of desired thickness. The slurry was spread evenly using a clean glass rod on to grease free glass plates placed side by side and fixed using leucoplast. The air dried plates were then activated in the oven at 110°C for 30 to 40 min and used as and when needed.
Standard graph for estimation of cadmium by dithizone method
Standard graph for estimation of cadmium by Atomic absorption spectroscopy
Standard graph for estimation of arsenate by volumetric method
Standard graph for estimation of arsenite by volumetric analysis
Standard graph for estimation of protein molecular weight