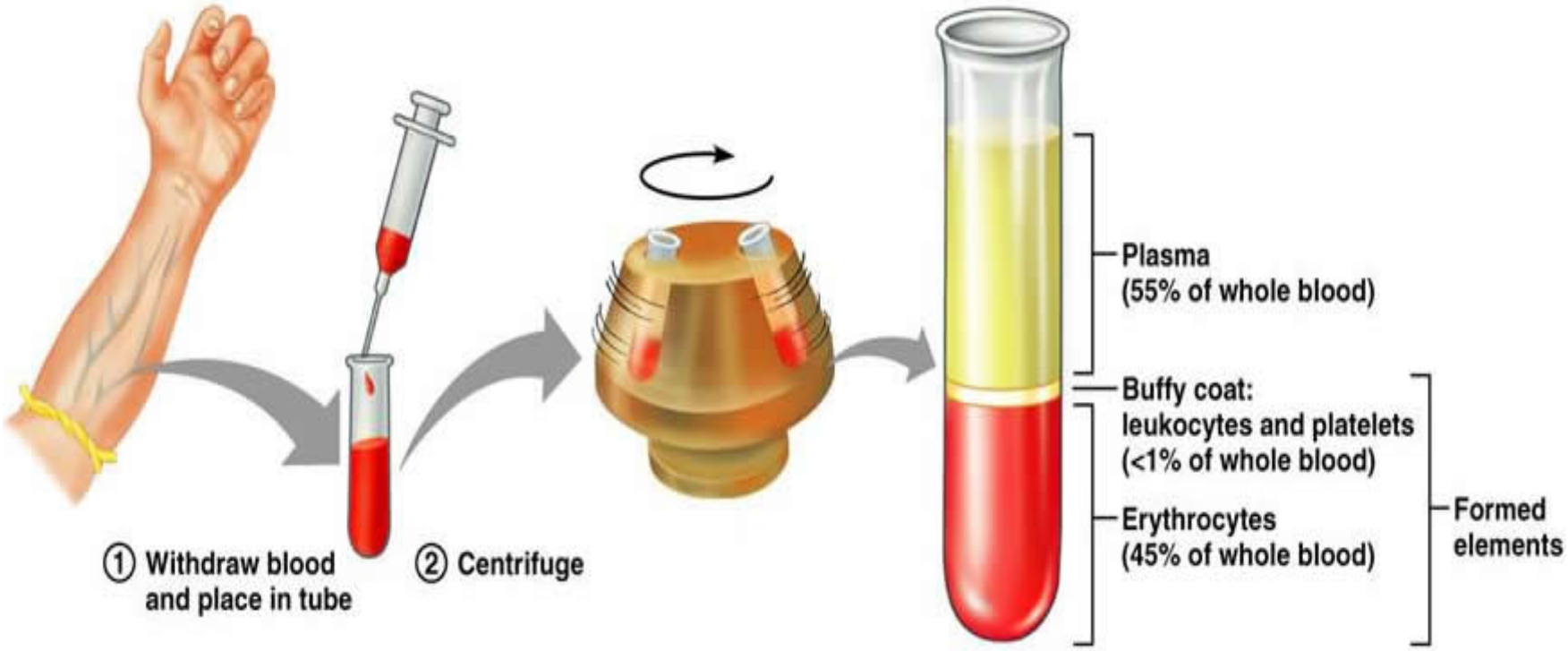
Genomic DNA Isolation from Blood

Prof Vandana Rai
Department of Biotechnology
VBS Purvanchal University
Jaunpur



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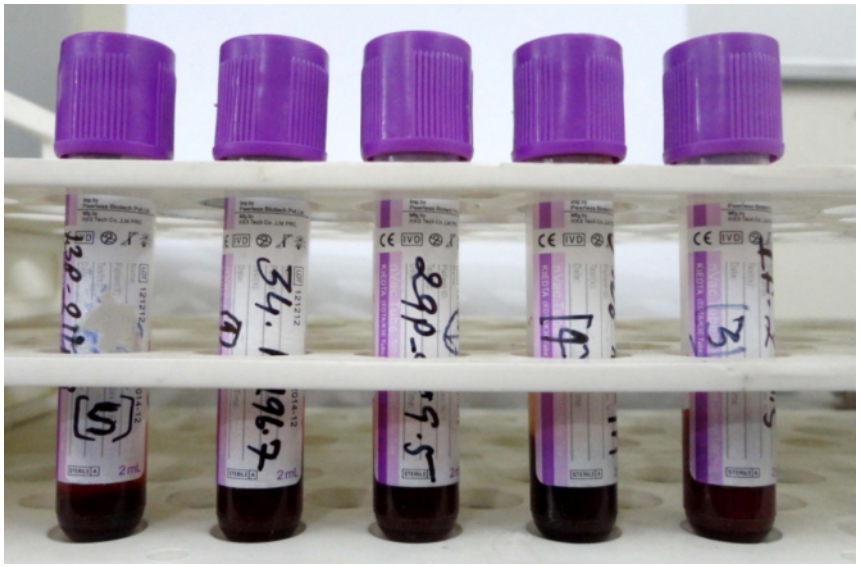
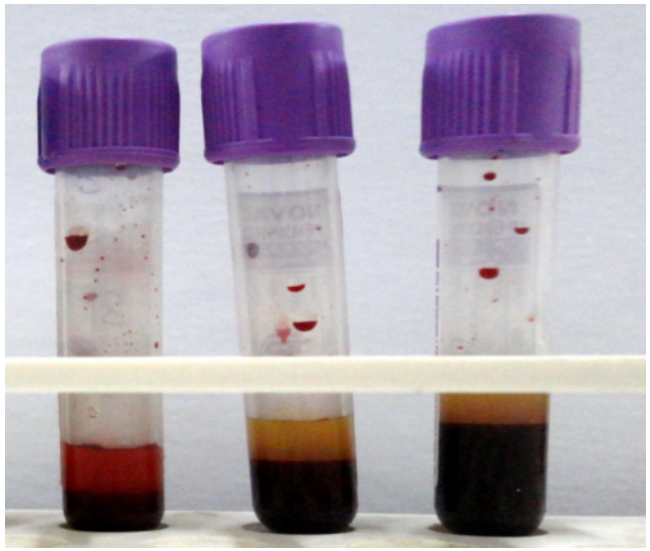
Blood Samples



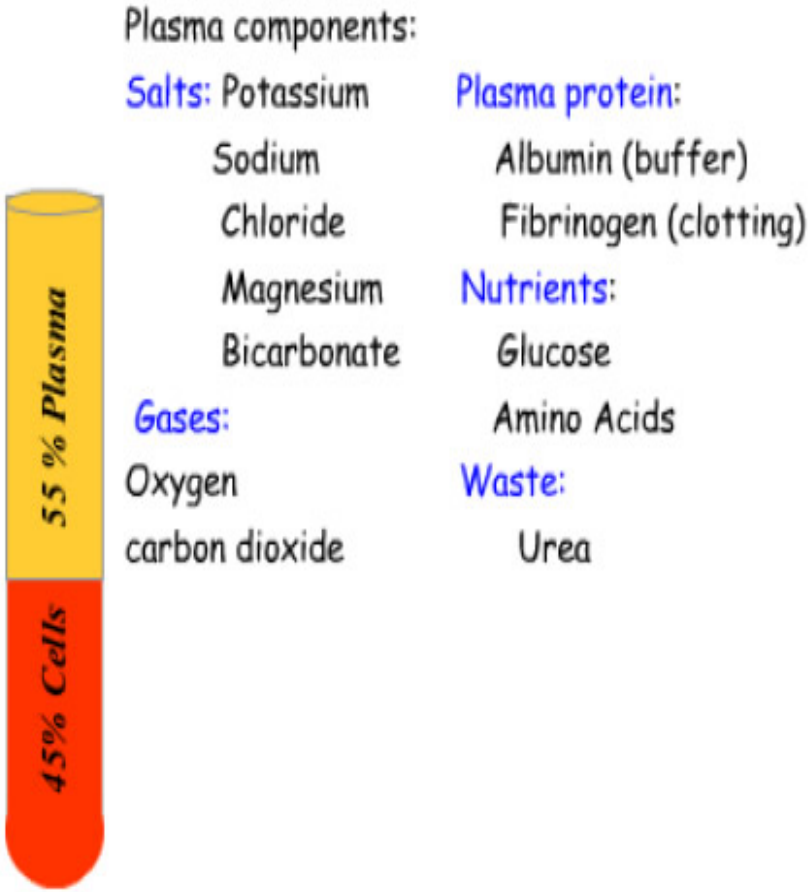


Blood Plasma Components- 55%

90% Water

8% Solutes:

- Proteins
  - Albumin (60 %)
  - Alpha and Beta Globulins
  - Gamma Globulins
  - fibrinogens
- Gas
- Electrolytes



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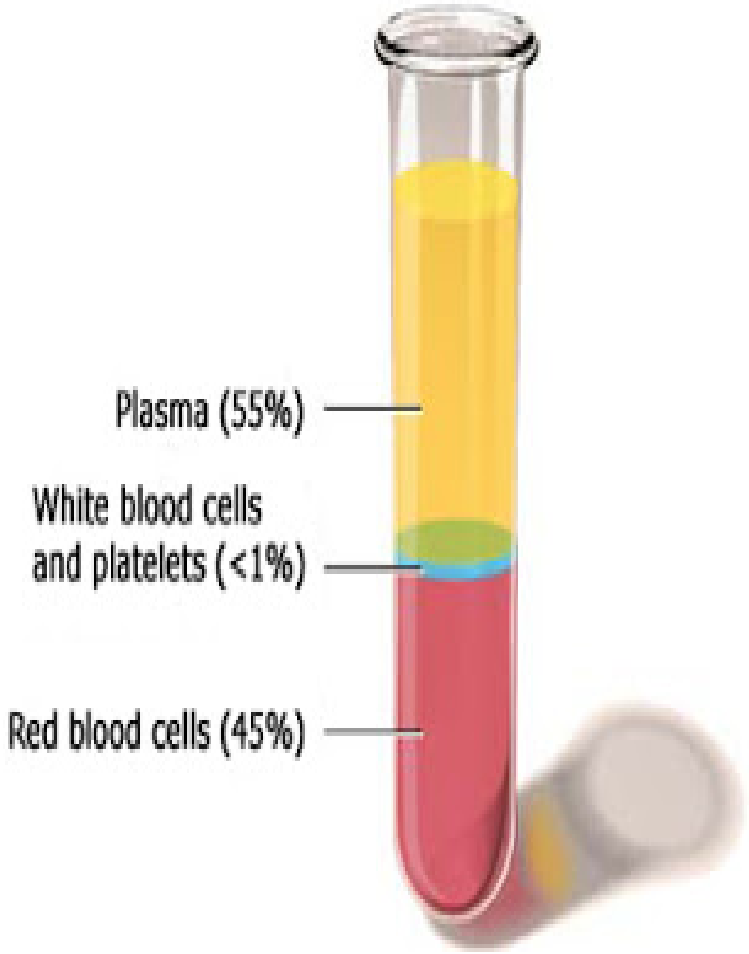
Plasma

- Plasma is a straw coloured, viscous fluid constituting nearly 55 per cent of the blood.
- 90-92 per cent of plasma is water and proteins contribute 6-8per cent of it.
- Fibrinogen, globulins and albumins are the major proteins.
- Fibrinogens are needed for clotting or coagulation of blood.



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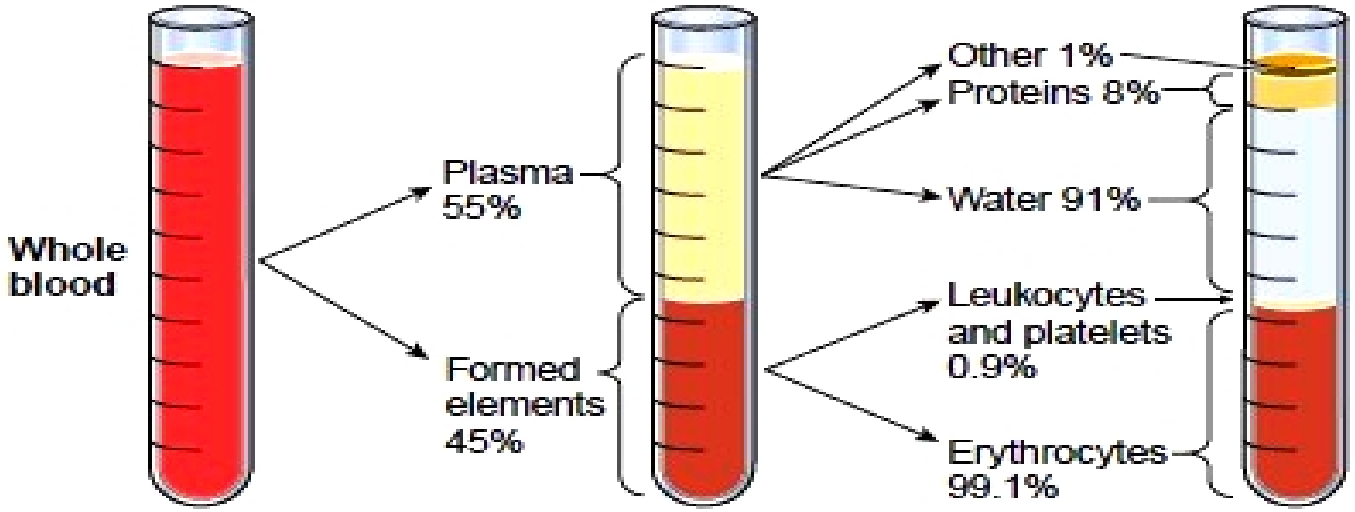
- Globulins primarily are involved in defense mechanisms of the body
- Albumins are help in osmotic balance.
- Plasma also contains small amounts of minerals like Na^{+}, Ca^{++}, Mg^{++}, $HCO3^{-}$, Cl^{-}, etc.
- Glucose, amino acids, lipids, are also present in the plasma as they are always in transit in the body.
- Factors for coagulation or clotting of blood are also present in the plasma in an inactive form.
- Plasma without the clotting factors is called serum. (NCERT Biology 11th)



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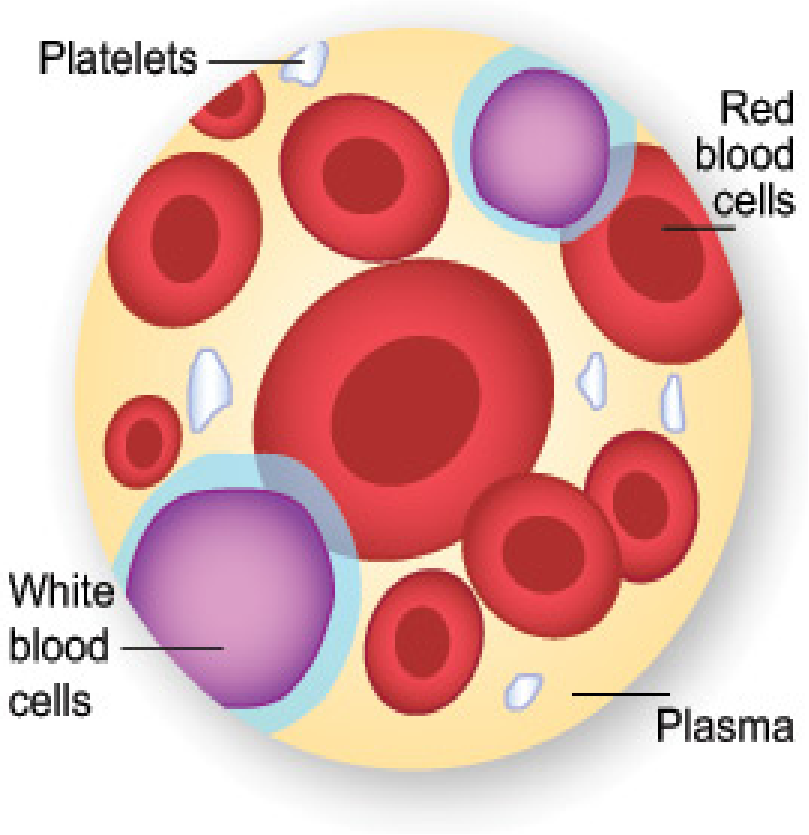
Formed Elements of the Blood- 45%

- Erythrocytes (red blood cells)
- Leukocytes (white blood cells)
- Platelets



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Erythrocyte
(7.8µm in diameter)

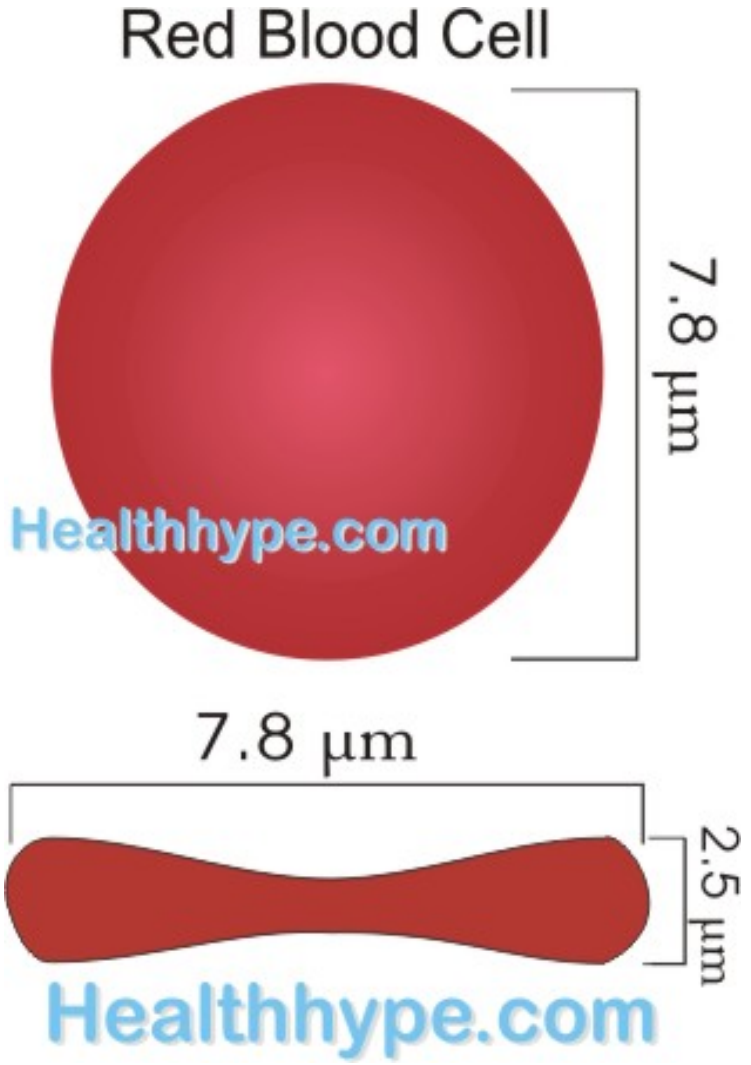
•Erythrocytes or red blood cells (RBC) are the most abundant of all the cells in blood.

•Normal red blood cells are biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center. (Guyton & Hall 11th edition)

•A healthy adult man has, on an average, 5 millions to 5.5 millions of RBCs mm^{-3} of blood.

•RBCs are formed in the red bone marrow in the adults.

•RBCs are devoid of nucleus in most of the mammals and are biconcave in shape.



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They have a red coloured, iron containing complex protein called haemoglobin, hence the colour and name of these cells.

A healthy individual has 12-16 gms of haemoglobin in every 100 ml of blood.

These molecules play a significant role in transport of respiratory gases.

RBCs have an average life span of 120 days after which they are destroyed in the spleen (graveyard of RBCs). (NCERT Biology 11th)

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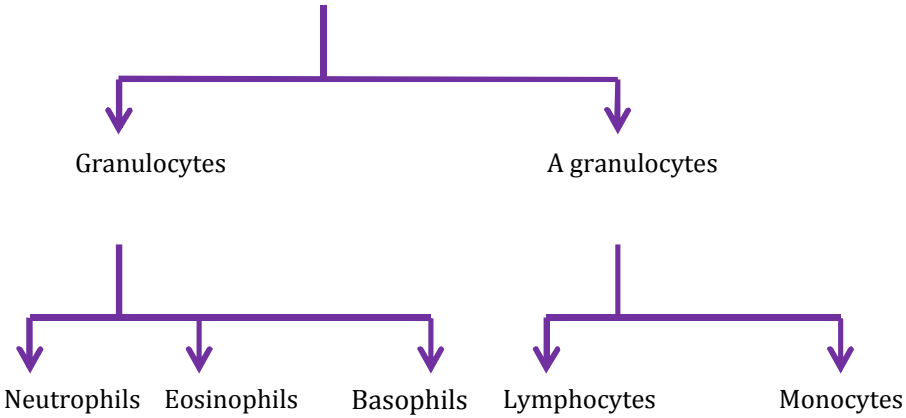
Leucocytes

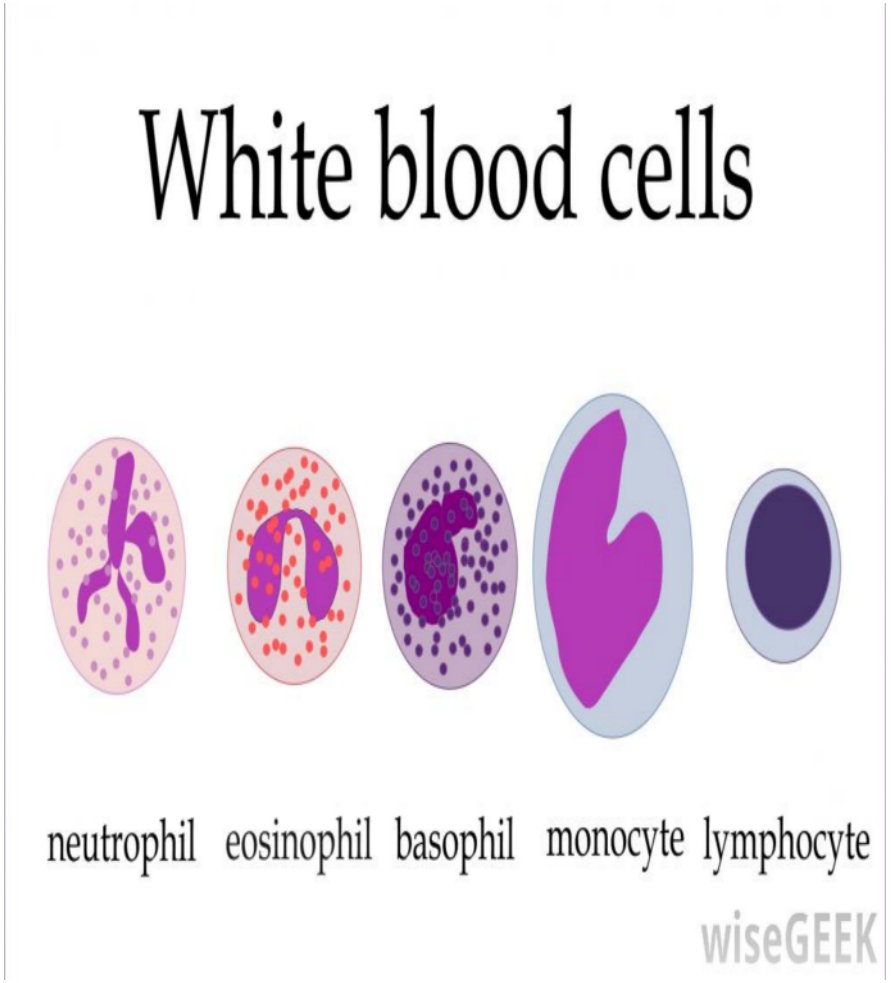
Leucocytes are also known as white blood cells (WBC) as they are colourless due to the lack of haemoglobin.

They are nucleated and are relatively lesser in number which averages 6000-8000 mm–3 of blood.

Leucocytes are generally short lived.

WBCs have two main categories. (NCERT Biology 11th)





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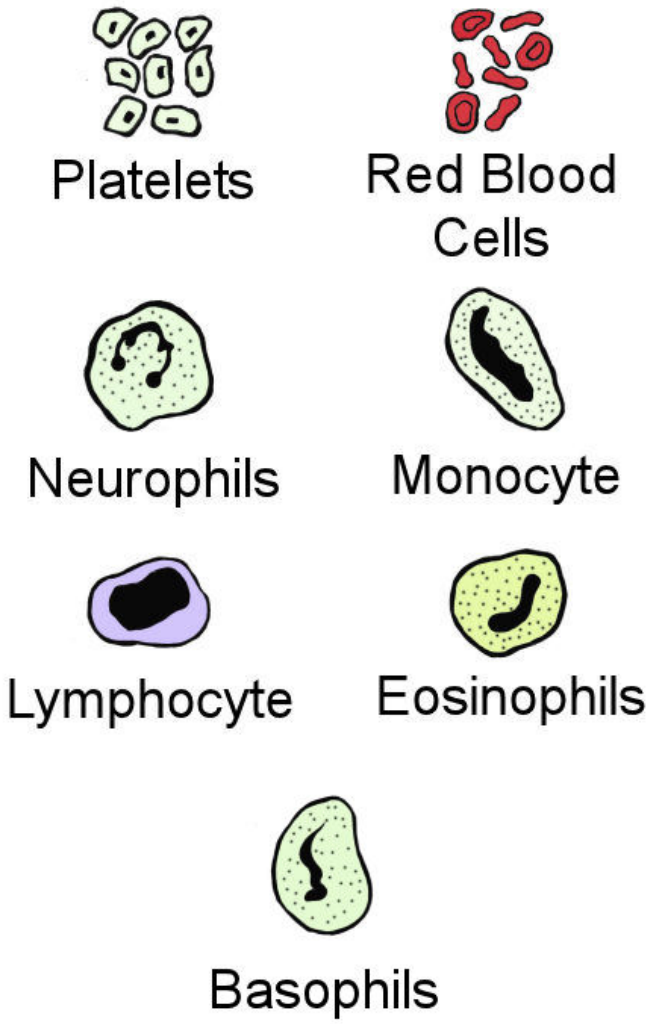
- Neutrophils are the most abundant cells (60-65 per cent) of the total WBCs.
- Basophils are the least (0.5-1 per cent)among them.
- Neutrophils and monocytes (6-8 per cent) are phagocytic cells which destroy foreign organisms entering the body.
- Basophils secrete histamine, serotonin, heparin, etc., and are involved in inflammatory reactions.
- Eosinophils (2-3 per cent) resist infections and are also associated with allergic reactions.



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- Lymphocytes (20-25 per cent) are of two major types – 'B' and 'T' forms.
- Both B and T lymphocytes are responsible for immune responses of the body.
- Platelets also called thrombocytes, are cell fragments produced from megakaryocytes (special cells in the bone marrow).
- Blood normally contains 1,500,00-3,500,00 platelets mm^{-3} .
- Platelets can release a variety of substances most of which are involved in the coagulation or clotting of blood.
- A reduction in their number can lead to clotting disorders which will lead to excessive loss of blood from the body. (NCERT Biology 11^{th})

- DNA is an extremely stable molecule, but enzymes which catalyses the breakdown of nucleic acids (nucleases) are found in all cells.
- In intact cells the DNA is found in the nucleus and thus is protected from the action of nucleases which are abundant in the lysosomes in the cytoplasm.
- Thus in order to isolate the genomic DNA from blood , the cell is first broken by opening of the cell membrane and nuclear membrane.
- However when cells are lysed, the membranes of cell compartments are disrupted, allowing nucleases to come in to contact with the DNA released from nucleus and degrade it.
- Thus the first stage of DNA extraction, uses buffers which contain inhibitors of nuclease activity.
- Lastly, the released DNA must be separated from other components such as the proteins, lipid, RNA, carbohydrates, amino acids, fatty acids and other cellular molecules.
- The purity of the DNA is essential as slight contaminants can inhibit further experiments like Restriction Enzyme digestion, Polymerase Chain Reaction, RFLP analysis, Southern blotting and leaving it unsuitable for the DNA quantification.

Requirements

- **Chemicals:**

  NaCl, Tris,$MgCl_2$,Triton X-100, Sucrose, EDTA, Sodium perchlorate, Chloroform, Ethanol, 70% ethanol,HCl(to maintain pH), NaOH(to maintain pH), Agarose, Ethidium bromide, Acetic acid, Loading dye etc.

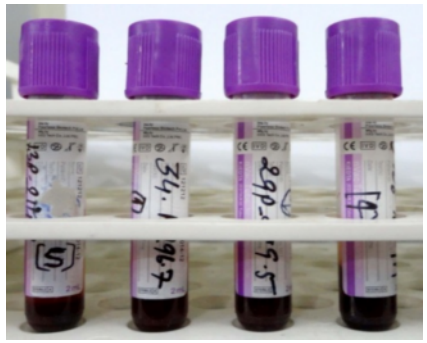
- **Plasticwares:**

  PW tubes, eppendorf and PCR tubes, micropippets (1 ml, T-200, T-20, T-2), microtips, racks (eppendorf rack,PCR tube rack, Plastic tray

  Tissue paper, Gloves

Genomic DNA Extraction from Human blood

About 3 ml of blood will be mixed with 6 ml (double volume of blood)of autoclaved 0.9%NaCl, incubate for 5 minutes and then centrifuge at 5000 rpm at room temperature



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Discard Supernatant without disturbing the pellet

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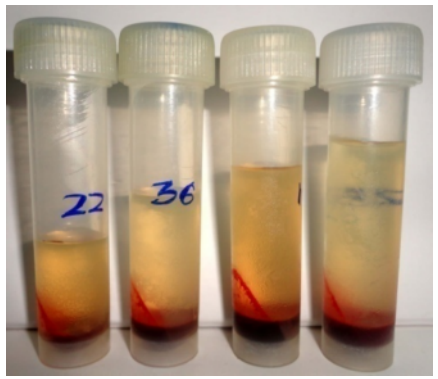
Break the pellet (loose), add chilled Reagent A hemolytic solution (four times of the blood), mix for 5 minutes and then centrifuge (5000rpm, 5 minutes)



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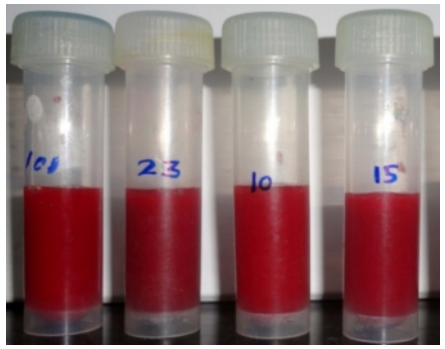
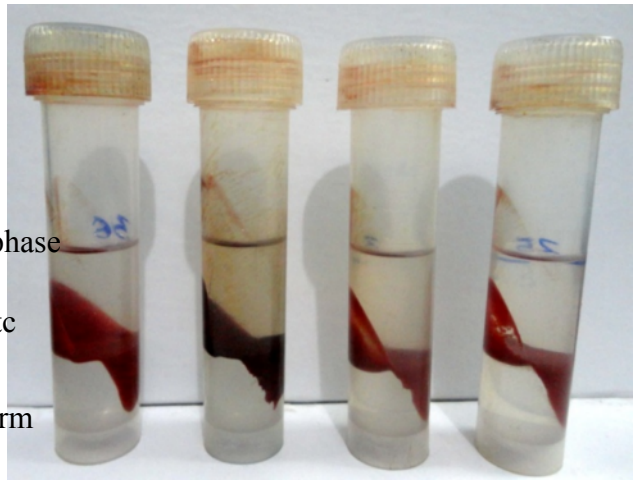
Discard Supernatant, and add 1.5 ml of Reagent B(room temperature) mix well for 5 min.

Add 0.5 ml of Reagent C (Sodium per chlorate) mix by inverting tube several times. Keep for 10-15 minutes.

Add 3 ml of chilled chloroform (volume similar to initial blood volume) and mix on rolling and rotating for 15-20 minutes.

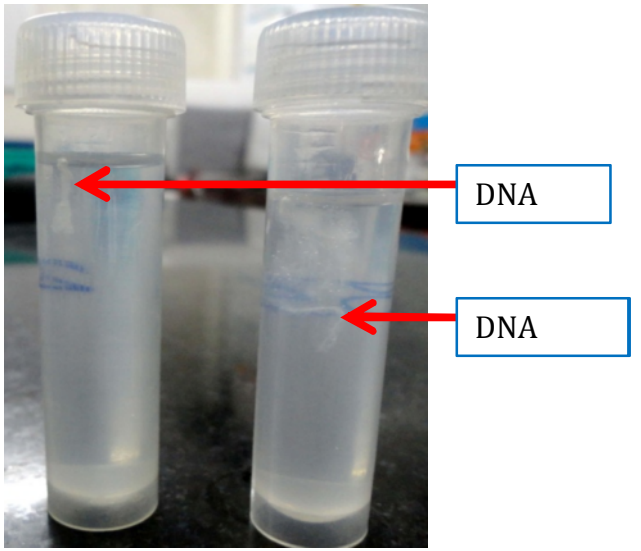


Centrifuge at 5000rpm for 5 min at 4^0 C.



Transfer upper aqueous layer in separate sterile tube, then add chilled ethanol (two volume of aqueous solution) and invert gently to allow DNA to precipitate

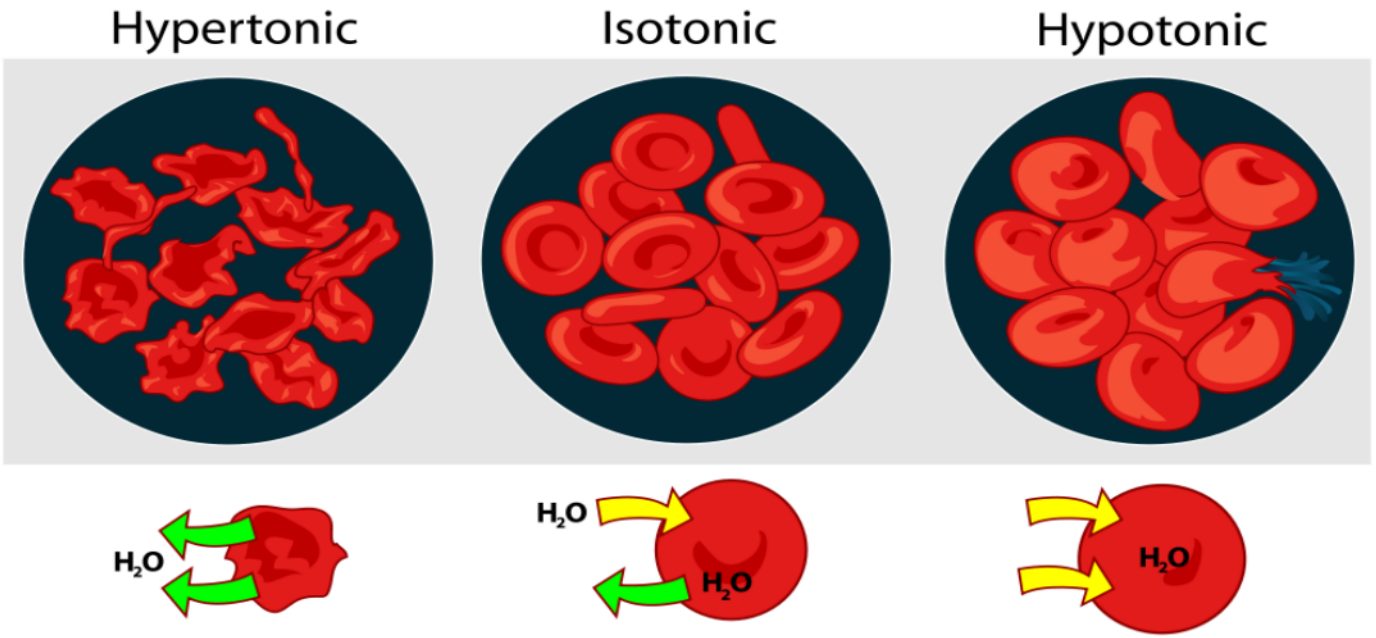
Transfer DNA to neweppendorf tube (by blunt end tip), wash twice with 70% alcohol. Dry Pellet in the incubator for 15 to 30 min at 37^0 C.



Dissolve in 150/200 ul of TE

Chemical Function

- .9% Nacl: it is a salt solution in which red cells neither swell nor shrink.
- .9% Nacl is a isotonic solution.
- Isotonic solution does not change the shape of red cells.
- An isotonic solution may be prepared with various types of salts; sodium chloride, calcium chloride, potassium chloride etc. (A laboratory manual human blood analysis, M.K.Bhasin)



osmotic-pressure-blood-cells
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REAGENT A (for 100 ml)

STOCK SOLUTION	WORKING SOLUTION	Volume in ml
1 M Tris Hcl (pH 7.4)	10 mM Tris Hcl	1 ml
1 M Sucrose	320 mM sucrose	32 ml
1 M $MgCl_2$	5mM $MgCl_2$	0.5ml
Triton X 100	1% Triton X 100	1 ml
	ddH_2O	65.5 ml

Tris: (2-amino-2-hydroxymethylpropane-1,3-diol)
Firstly it's used to get the right pH for DNA extraction, but Tris is preffered over other buffers because Tris interacts with the lipopolysaccharides present on the outer membrane which helps to permeabilize the membrane. This effect is enhanced with the addition of EDTA thylenediaminetetraacetic acid), which is a chelating agent that captures metal ions (like mg^{2+}).

http://www.thenakedscientists.com/forum/index.php?topic=26245.0

Tris has a very high buffering capacity, is highly soluble in water, and is inert in the wide variety of enzymatic reactions. (Molecular Cloning , Sambrook and Russell 3rd edition)

- **Sucrose:**
- Sucrose provides osmotic balance into and outside the cell.
- Sucrose prevents the cells from bursting immediately. (T.A.Brown 6th edition)

- **$MgCl_2$**:
- When membranes are busted by TRIS, there is no compartmentalization in the solution anymore.

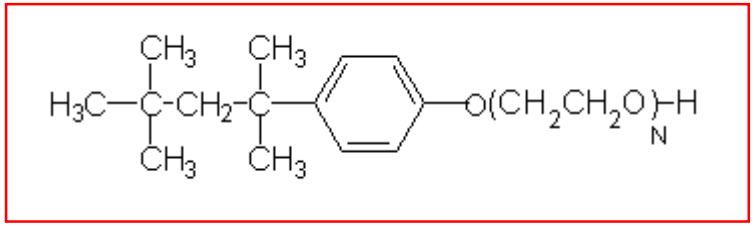
- $MgCl_2$ is then used because it binds to DNA and thus protects it against DNase proteins that are now (because of lack of membranes) in direct contact with your DNA.

- The binding of $MgCl_2$ to DNA denies access of DNase to the DNA, and your DNA will not be broken down.

http://www.thenakedscientists.com/forum/index.php?topic=26245.0

Triton X-100

- Triton X-100 is a non-ionic detergent which is induced Cell lysis. (T.A. Brown 6th edition)
- It is often used in biochemical applications to solubilize proteins.
- It has no antimicrobial properties.



- Triton X-100 is soluble in all proportions at 25°C in water. (Sigma Product Information Sheet)

REAGENT B (for 100 ml)

(White Cell Lysis Buffer)

STOCK SOLUTION	WORKING SOLUTION		Volume in ml
1M Tris HCl	400 mM Tris HCl	40 ml	
O.5M EDTA	60 mM EDTA		12 ml
5M NaCl	150 mM NaCl		03 ml
20 % SDS	1 % SDS	01 ml	
	ddH_2O		44 ml

EDTA:

- EDTA is a chelating agent which chelate divalent cations such as $Mg^{2+}$and thereby to inhibit the action of any residual nucleases that degrade DNA. (Molecular Cloning, Sambrook and Russell 3rd edition)

- EDTA (ethylenediamine tetraacetate) removes magnesium ions that are essential for preserving the overall structure of the cell envelope, and also inhibits cellular enzymes that could degrade DNA. (T.A.Brown 6th edition).

- **Nacl:**
- NaCl provides Na^+ ions that will block negative charge from phosphates on DNA.

- Negatively charged phosphates on DNA cause molecules to repel each other.

- The Na+ ions will form an ionic bond with the negatively charged phosphates on the DNA, neutralizing the negative charges and allowing the DNA molecules to come together.

http://answers.yahoo.com/question/index?qid=20080715225851AAOyfDD

SDS

- Sodium dodecyl sulphate (SDS) is a anionic detergents which cause lysis by removing lipid molecules and thereby cause disruption of the cell membranes. (T.A.Brown 6th edition)

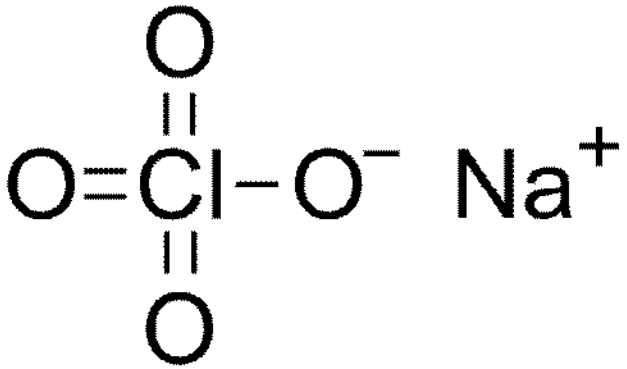
- SDS (Sodium dodecyl sulfate) is toxic, an irritant, and poses a risk of severe damage to the eyes. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles. Do not breathe the dust. do not autoclave. (Molecular Cloning Sambrook and Russell 3rd edition)

Reagent C
(Sodium per chlorate)

- **5M Sodium perchlorate:**
  35.115gm Sodium perchlorate dissolved in 50ml double distilled water.

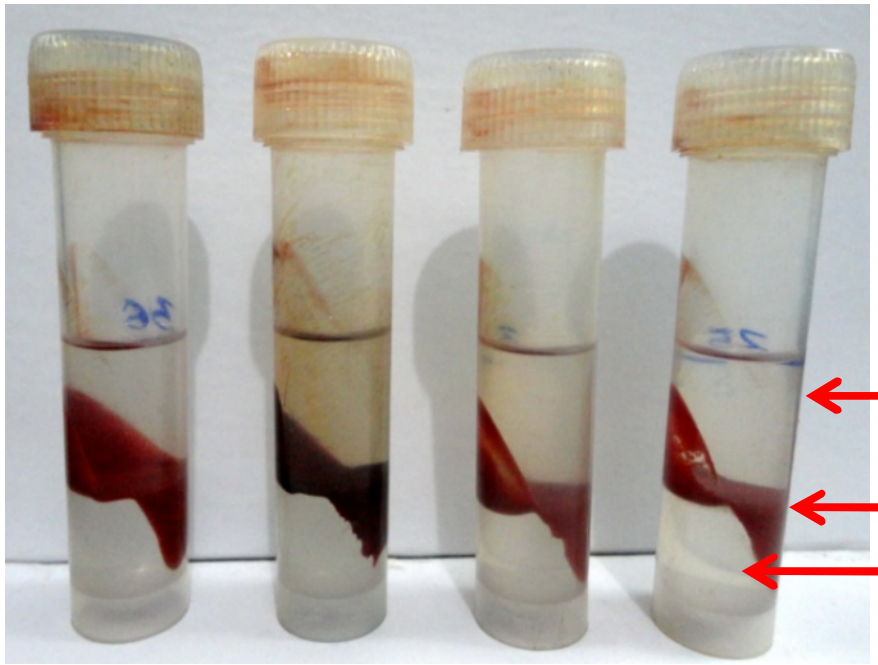
❖ Sodium perchlorate in high concentrations will remove from solution the detergent sodium dodecyl sulphate and protein complexed with it.

❖ This and the failure of proteins to be precipitated by ethanol from solutions containing a high concentration of sodium perchlorate can be utilized as efficient, rapid and simple deproteinization procedures during the preparation of nucleic acids. JOHN WILCOCKSON (1973); Biochem. J. 135, 559-561



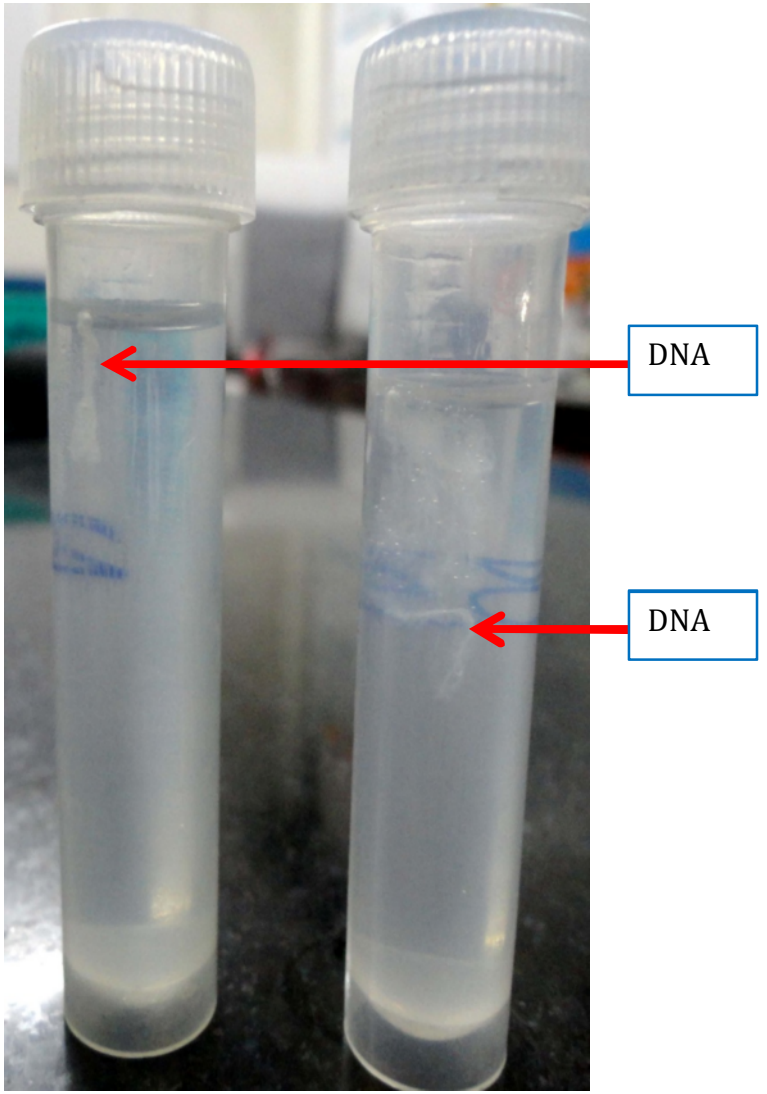
Chloroform

- The chloroform denatures proteins and facilitates the separation of the aqueous and organic phases,
  (Molecular Cloning, Sambrook and Russell 3rd edition)



Ethanol Precipitation

- Precipitation with ethanol is the standard method to recover nucleic acids from aqueous solutions.
- Ethanol depletes the hydration shell from nucleic acids and exposes negatively charged phosphate groups.
- Counter ions such as Na+ bind to the charged groups and reduce the repulsive forces between the polynucleotide chains to the point where a precipitate can form.
- Ethanol precipitation can therefore only occur if cations are available in sufficient quantity to neutralize the charge on the exposed phosphate residues. (Molecular Cloning, Sambrook and Russell 3rd edition)



TE buffer

TE Buffer (50ml)

Stock Concentration	Working Concentration	Volume
1M Tris-Cl (pH-7.5)	10mM	500 ul
1M EDTA (pH-8.0)	1mM	100 ul
dd water		49.4 ml