1.1 Lab Skills

Lab Hazards

<u>Harm</u> caused to an individual when working in a laboratory

Types of Lab Hazards

1. Toxic or corrosive chemicals
2. Heat or flammable substances
3. Pathogenic organisms
4. Mechanical equipment.

Risk

The **likelihood** of harm arising from exposure to a hazard.

Risk assessment

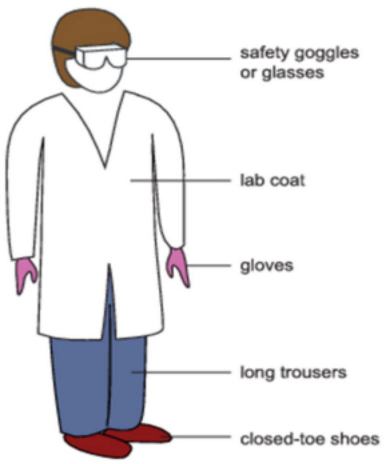
Involves identifying control measures to **minimise** the risk.

Control measures

Measures aim to reduce risk of harm caused by a hazard.

Types

1. Appropriate handling technique
2. Protective clothing and equipment
3. Aseptic technique.



Standard laboratory PPE

1.2 Dilution

Linear Dilution Series

Differ by an equal interval

E.g 0·1M, 0·2M, 0·3M

Flask	Inhibitor volume (cm^3)	Glucose volume (cm^3)	FBS volume (cm^3)	Buffer volume (cm^3)	Final inhibitor concentration (%)
1	0·00	1·00	5·00	19·00	0
2		1·00	5·00		20

Logarithmic Dilution Series

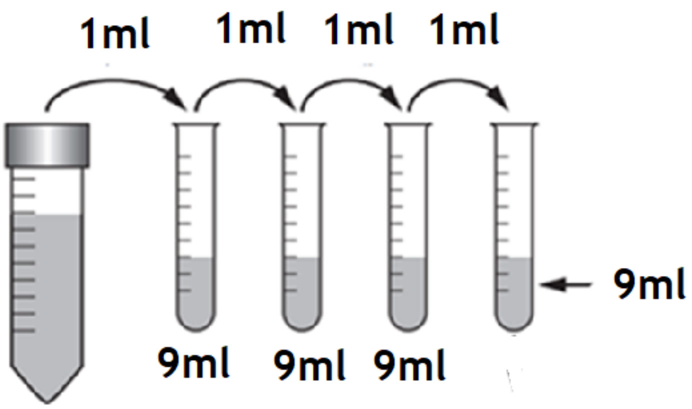
Differ by a constant proportion

E. g 10^{-1}, 10^{-2}, 10^{-3}

Serial Dilution

Taking 1 ml of a stock solution and adding this to 9ml of water. Then 1ml of the newly diluted solution is extracted and subsequently added to 9ml of water each time.

This dilutes the solution by a factor of 10 each time creating a logarithmic dilution series.



1.3 Spectrophotometer/Colorimeter

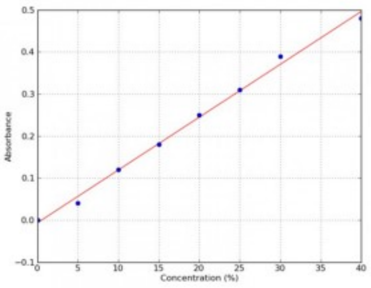
Concentration of Unknown solution

Measured by a device called a spectrophotometer OR colorimeter by measuring the

absorbance/transmission of a solution at a certain wavelength of visible light.

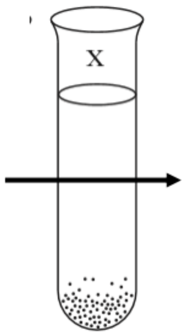
Absorbance

The more intense the colour (concentration) the higher the absorbance .



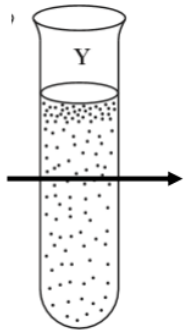
Transmission

As turbidity increases, less light passes through the sample and the transmission value is lower.



Low turbidity

High transmission value



High turbidity

Low transmission value

Calibration

Calibration via a colourless solution should always be carried out to ensure the machine is working correctly.

Absorbance values 0.00

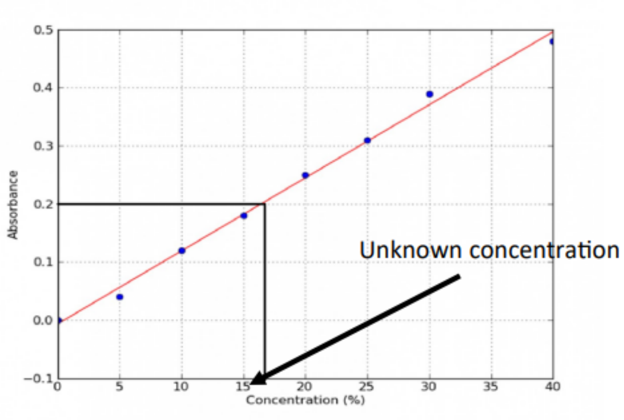
Transmission 100%

1.3 Spectrophotometer/Colorimeter

Standard Curve

A standard curve can be created by measuring known concentrations of solution and taking readings to plot a line of best fit.

An unknown concentration can then be read off this to ascertain the unknown concentration.

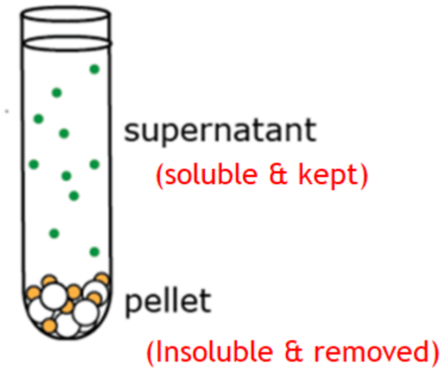


1.4 Separating techniques: Centrifugation

Centrifuge

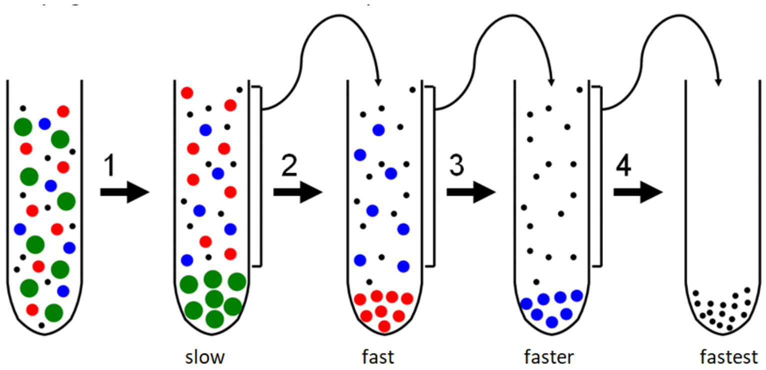
To separate substances of differing **density**

1. **More dense** components settle in the **pellet**
2. **Less dense** components remain in the **supernatant.**



Increasing speed/time in successive centrifuge cycles

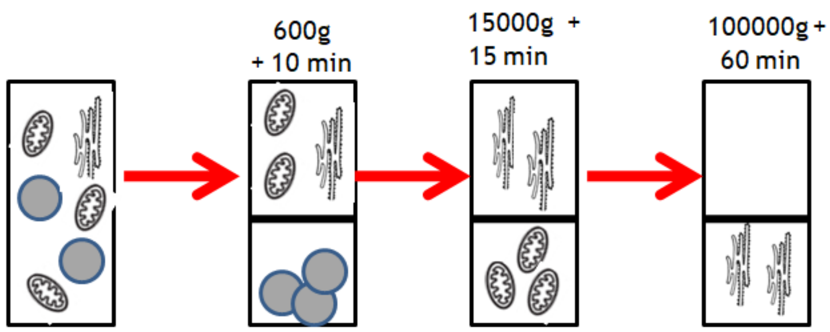
Less dense material moves down into pellet
Only lightest molecules are left in supernatant.



To separate cell organelles

Higher speed/force is applied repeatedly for longer time periods to separate increasing less dense organelles

Step	Centrifuge conditions		Cell component(s) in pellet
	Force (g)	Time (minutes)	
1	600	10	nucleus, cytoskeleton
2	15 000	15	mitochondria
3	100 000	60	plasma membrane, endoplasmic reticulum fragments



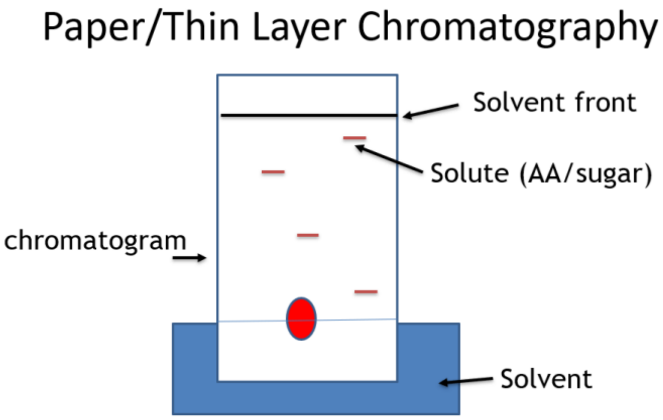
1.4 Separating techniques: Chromatography

Chromatography

1. Paper and thin layer chromatography

To separate substances such as amino acids or sugars.

The speed that each solute travels along the chromatogram depends on its differing solubility in the solvent used.

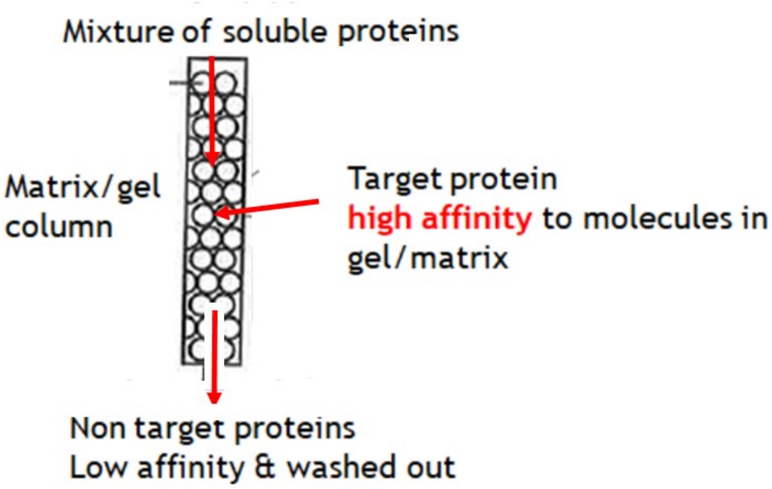


2. Affinity Chromatography

A solid matrix or gel column is created with specific molecules bound to the matrix/gel and mixture of proteins passed down column.

1. Soluble, **target proteins** with a **high affinity** molecules bound to gel/matrix become attached to them.

2. **Non-target** molecules with a **weaker affinity** are washed out



1.4 Separating techniques: Gel Electrophoresis

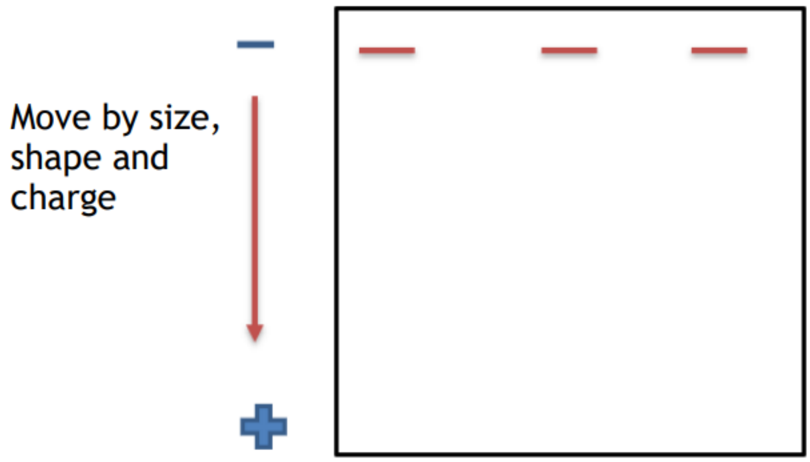
Gel Electrophoresis

Charged macromolecules move through an electric field applied to a gel matrix.

Native Gel Electrophoresis

Separates proteins by their **shape, size and charge**

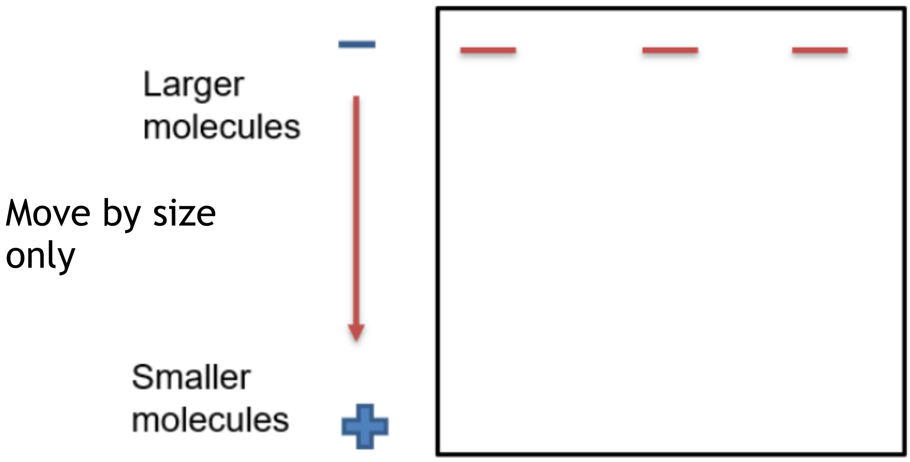
The proteins are not denatured in this technique.



SDS Page Electrophoresis

Separates proteins by **size alone** (smaller molecules travel further).

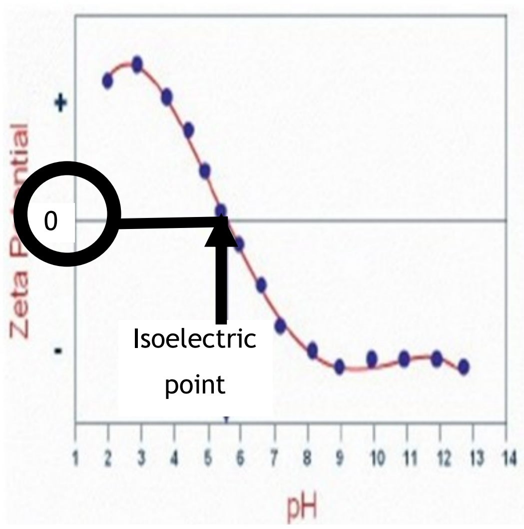
The proteins are denatured giving an equally negative charge



1.4 Separating techniques: Gel Electrophoresis

Iso electric Point

When there is **no net charge** on the protein and it will precipitate out of solution.



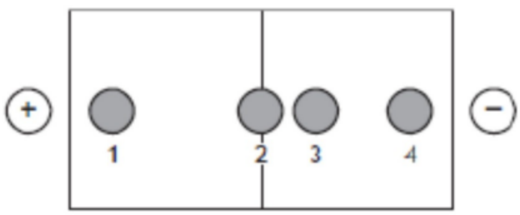
Isoelectric Focusing

Soluble proteins separated using their IEP in electrophoresis using an electric field and a pH gradient.

A protein stops migrating through the gel at its IEP in the **pH gradient because it has no net charge** and will **precipitate** out of solution.

Buffers required: creating a pH gradient in the gel matrix

Holds the pH constant despite adding small quantities of acid or alkali.



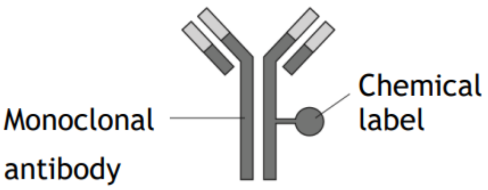
1.5 Immunoassay

Immunoassay techniques

Used to detect and identify specific proteins using monoclonal antibodies

Monoclonal antibodies

Stocks of antibodies with the same specificity to a particular antigen.



Types of chemical labels

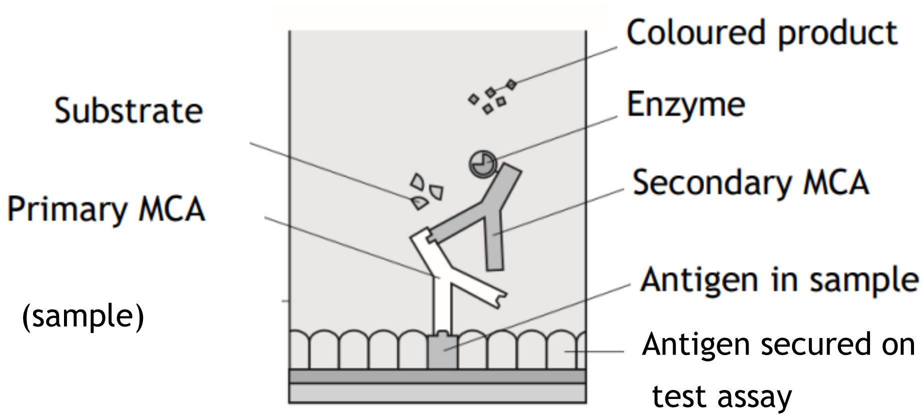
1. Reporter enzyme producing a colour change (ELISA technique)
2. Chemiluminescence Reporters
3. Fluorescence Reporters

Immunoassay e.g. ELISA

A MCA specific to the protein antigen is linked to a chemical 'label' to detect the presence/concentration of protein in the sample.

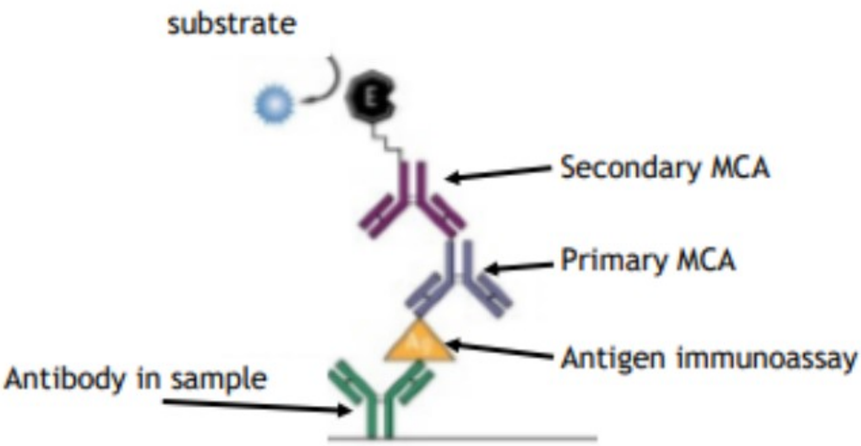
Used to detect the **presence/concentration** of antigen protein in a sample

Sometimes more than one MCA is used with the secondary MCA containing the chemical label.

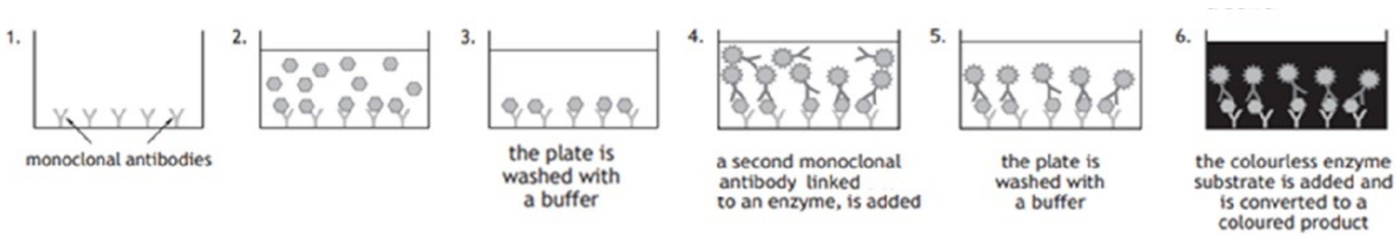


1.5 Immunoassay

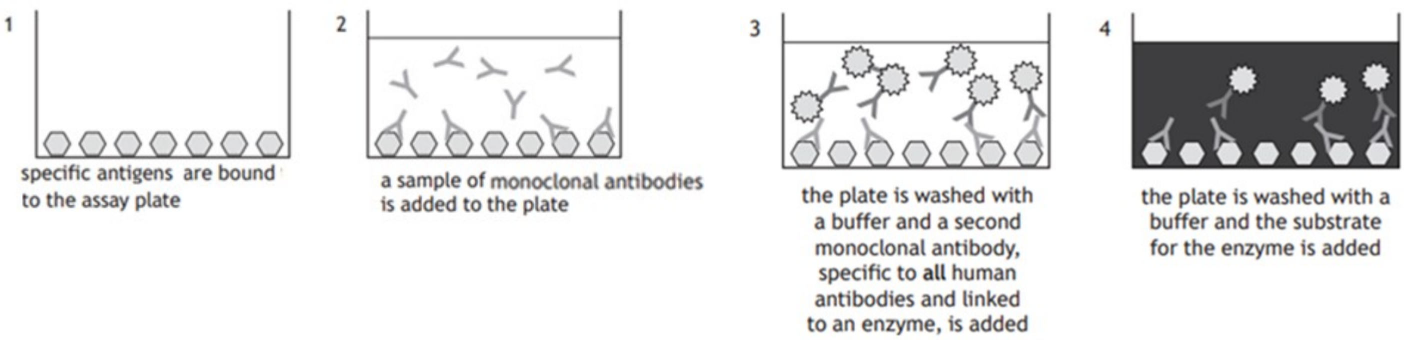
In some immunoassays, a **specific antigen** is used to detect the presence of antibodies rather than MCA's detecting specific antigens.



Stages of ELISA—MCA on test assay

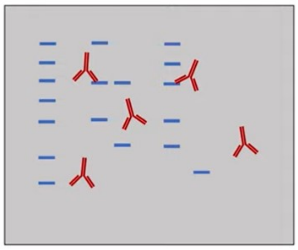


Stages of ELISA—Specific antigen on test assay



1.5 Western Blot

Allows a **single protein** to be identified from a **complex mix** using fluorescent labelled antibodies.

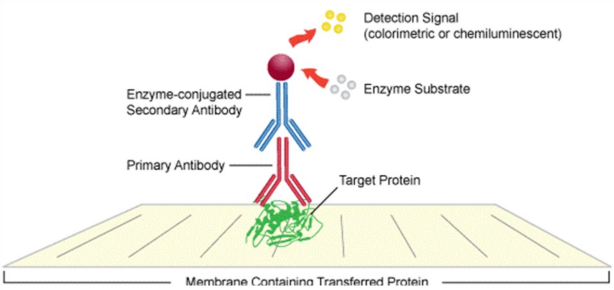


1. Separate a protein from a complex mix using **SDS-PAGE electrophoresis**

2. Probe for protein

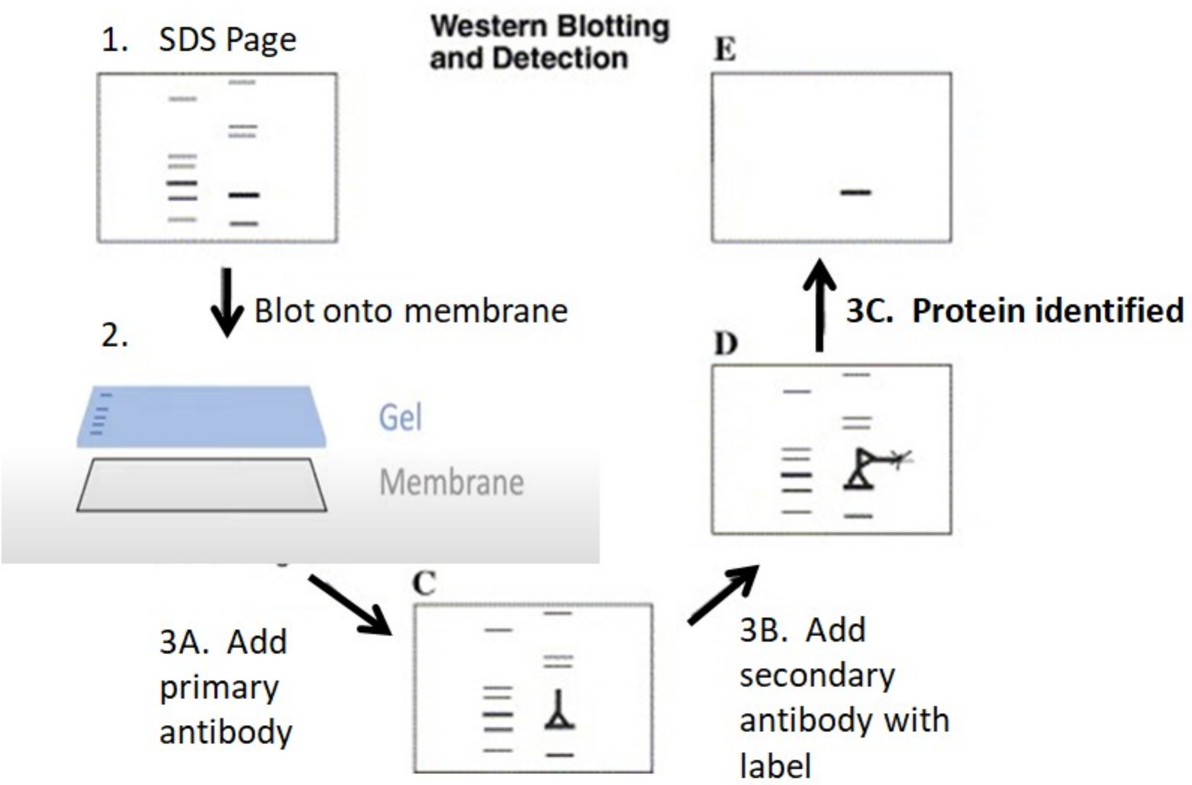
   The separated proteins from the gel are transferred (blotted) onto a solid medium

   The proteins are identified using **specific antibodies** that have reporter enzymes attached.



Nylon membrane containing transferred protein

Stages of Western Blot



1.6 Microscopy

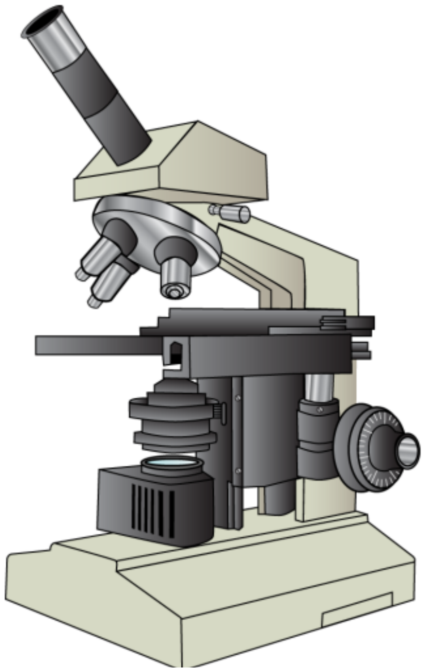
1. Bright Field Microscopy
   Allows the following to be viewed:

   (a) Whole organisms

   (b) Parts of organisms

   (c) Thin sections of dissected tissue

   (d) Individual cells



2. Fluorescence Microscopy

   Uses specific **__fluorescent labels__** to bind to and visualise certain molecules or structures **__within cells/tissues.__**

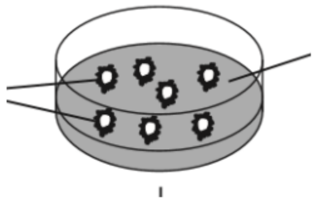
   Visualise structures that are **__much smaller__** in size (higher resolution).

1.7 Microbiology

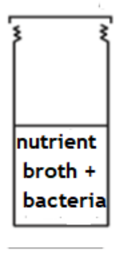
Types of Cell culture

A microbial culture can be started using an inoculum of microbial cells on:

1. Agar (solid media)



2. Broth (liquid media)



Nutrients in Cell Culture

Culture media contains suitable **nutrients** that promote the growth of **specific** cells/ microbes.

Example

Animal cells grown in media containing **growth factors** from **serum**.

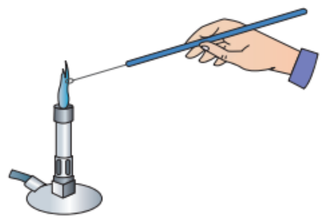
Growth factors are **proteins** that promote **cell growth** and **proliferation**.

Aseptic technique

Involves the **sterilisation** of equipment/culture media by **heat or chemical** means when carrying out cell culture experiments.

Examples

1. Wash hands & desk with chemical disinfectant.
2. Autoclave all glassware before use at high temperature/pressure
3. Work near the blue flame of a Bunsen burner (heat)
4. Flame the neck of all bottles/loop (heat)



Why are aseptic techniques necessary?

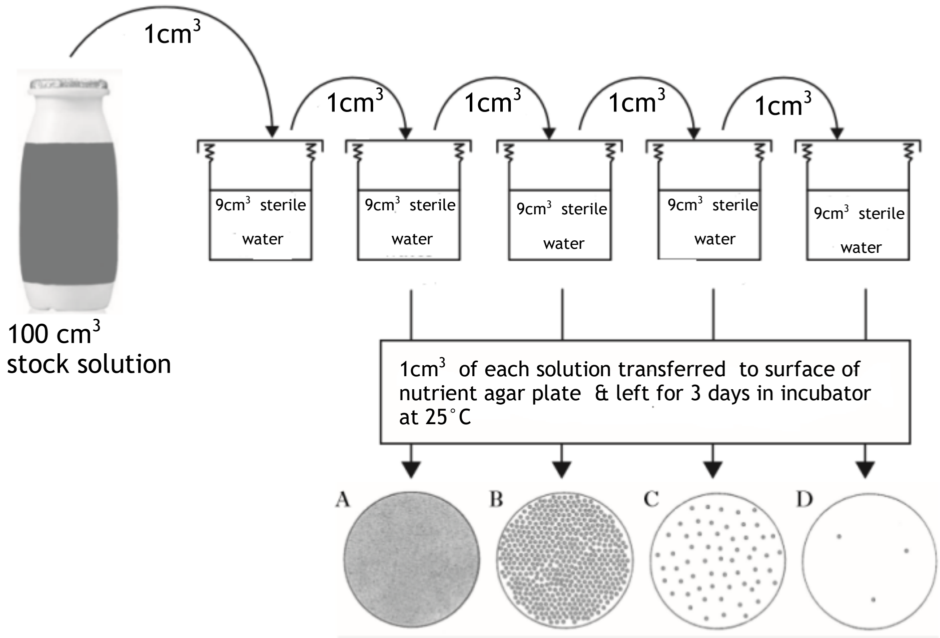
Eliminates unwanted **microbial contaminants** when culturing microorganisms /cells

1.7 Microbiology

1. Colony forming units (CFU)

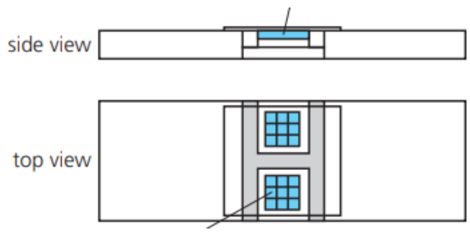
Plating out of a **liquid microbial culture** on solid media allows the number of CFU to be counted and the **density of cells** in the culture estimated.

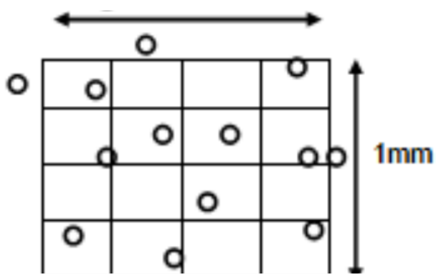
Serial dilution is often needed to achieve a suitable colony count. **(30-300 colonies)**



2. Haemocytometers

Use of haemocytometer to estimate cell numbers per cm^3 in a liquid broth culture





Step 1 Work out volume of liquid placed in haemocytometer slide in cm^3.

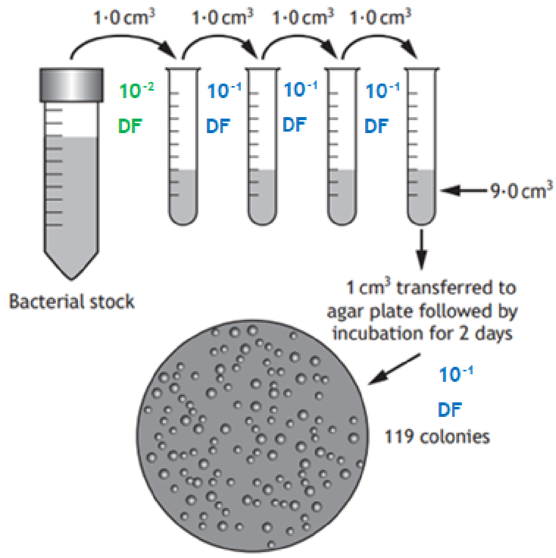
Length x breadth x depth (remember if if mm divide by 10)

Step 2 Count number of cell colonies viewed in diagram (microscope).

Step 3 Proportion calculation to scale up per cm^3.

1.7 Microbiology CFU Calculation

Worked Example 1



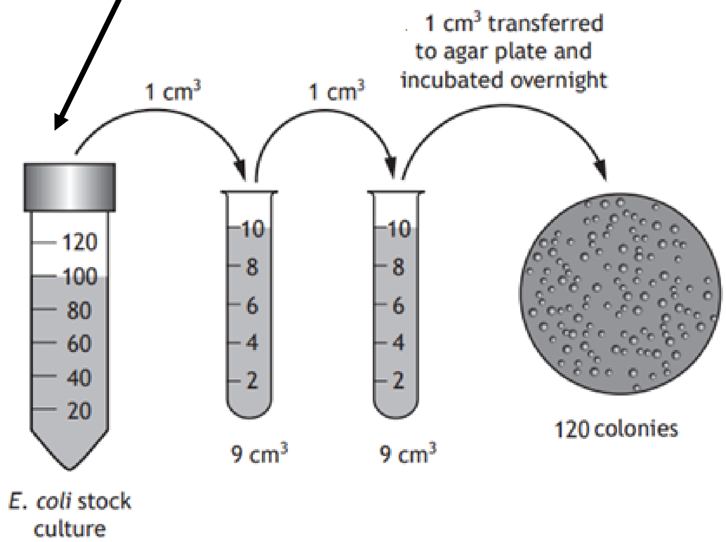
Total dilution factor 10^{-2} DF + 10^{-1} DF + 10^{-1} DF + 10^{-1} DF + 10^{-1} DF = 10^{-6} DF

DF 10^{-6} solution ⟶ 119 colonies

Original stock ⟶ 119×10^{6} = 119 000 00/ 1.19×10^{8} colonies

Worked Example 2

The figure shows how a biologist used serial dilution followed by plating to estimate the number of cells in a 100 cm^3 stock culture of *E.coli*.



How many *E.coli* cells were present in the original stock culture?

Total dilution factor 10^{-2} DF + 10^{-1} DF + 10^{-1} DF = 10^{-4} DF

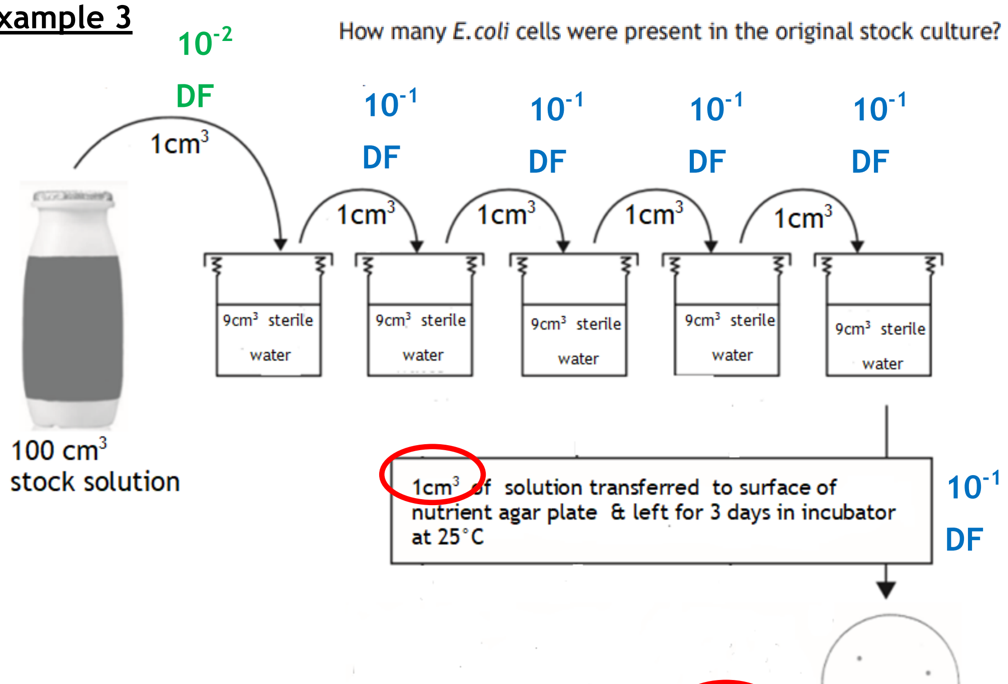
DF 10^{-4} solution ⟶ 120 colonies

Original stock ⟶ 120×10^{2} = 1200 000 / 1.2×10^{6} colonies

1.7 Microbiology CFU Calculations

Worked Example 3

How many *E.coli* cells were present in the original stock culture?



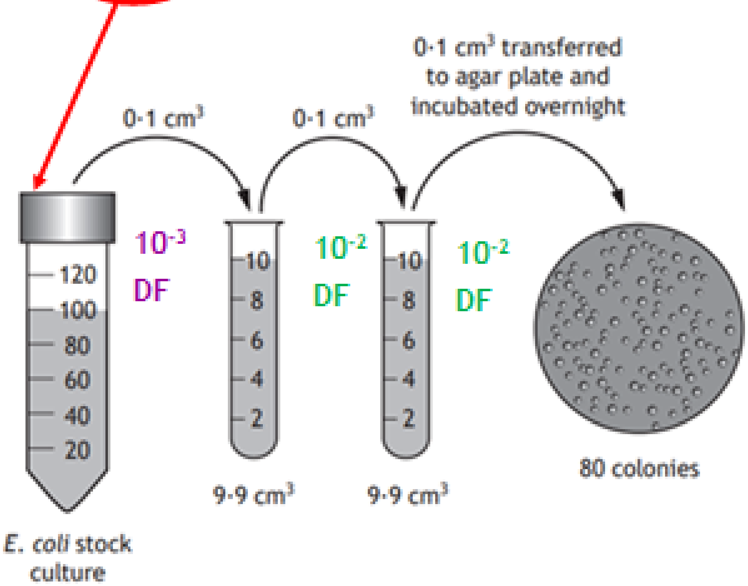
Total dilution factor 10^{-2} DF + 10^{-1} DF + 10^{-1} DF + 10^{-1} DF + 10^{-1} DF + 10^{-1} DF $= 10^{-7}$

DF 10^{-7} solution → 3 colonies

Original stock → 3×10^7 = 30 000 000/ 3×10^7 colonies

Worked Example 4

The figure shows how a biologist used serial dilution followed by plating to estimate the number of cells in 100 cm^3 stock culture of *E.coli*.



How many *E.coli* cells were present in the original stock culture?

Total dilution factor 10^{-3} + 10^{-2} + 10^{-2} = 10^{-7}

DF 10^{-7} solution → 80 colonies

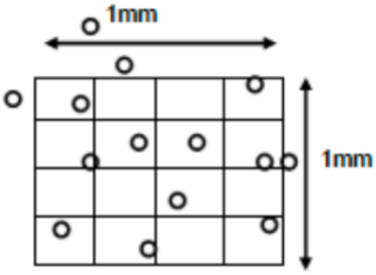
Original stock → 80×10^7 = 80 000 000/ 8×10^7 colonies

1.7 Microbiology Haemocytometer Calculations

Worked Example 1

The diagram below represents red blood cells in a haemocytometer. The grid is **0.1mm** in depth.
Calculate the number of red blood cells per cm^3 of the liquid culture.

1/10 = 0.1cm



1/10 = 0.1cm

A—

1.Work out Depth

L x b x h = 0.1 x 0.1 x 0.01 = **$0.0001cm^3$**

2.Count Cells

10 RBC's are on the inside of the haemocytometer grid.

3.Proportion $0.0001cm^3 \longrightarrow$ 10 RBC

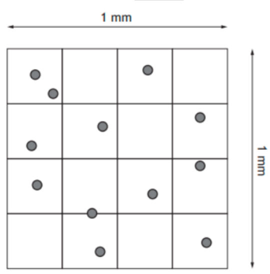
$1cm^3$ 3 $\longrightarrow$ **100,000 RBC**

Worked Example 2

A haemocytometer can be used to estimate the number of bacterial cells in a liquid culture. The figure represents bacterial cells from a culture, placed in a haemocytometer that has a depth of **0.1 mm**.

Calculate the number of bacterial cells per cm3 in a liquid culture.

1/10 = **0.1cm**



1/10 = **0.1cm**

1. Work out volume

L x b x h = 0.1 x 0.1 x 0.01 = **$0.0001cm^3$**

2.Count Cells

12 bacterial cells are on the inside of the haemocytometer grid.

3.Proportion

$0.0001cm^3 \longrightarrow$ 12 RBC

$1cm^3 \longrightarrow$ **120,000 bacterial cells**

1.7 Microbiology

Vital Staining of Cells

Measuring **viable** cell count.

Use of stains such as trypan/methylene blue that **only stain dead cells** as viable cells remove blue dye by active transport.