Southern blotting and Western blotting

Dr. Sudha Kumari

Department of Veterinary Microbiology
Bihar Veterinary College,Patna
Bihar Animal Sciences University , Patna

- ❖What is blotting technique
- ❖Types of blotting
- ❖Mechanism of southern and western blotting
- ❖Steps of southern and western blotting
- ❖Application of southern blotting and western blotting

Blotting

blot

membrane

Blotting

Absorption

What is blotting technique

❖ Blotting is a technique for **detecting DNA, RNA or proteins initially present in a complex mixture**. In this technique the molecules separated by
Gel electrophoresis

❖ Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge.

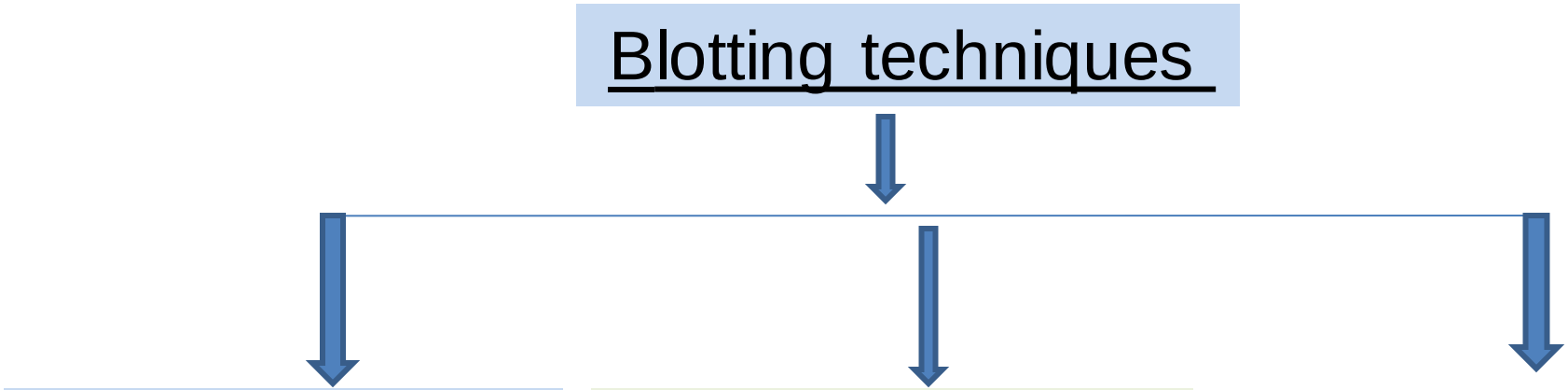
❖After the blotting, the transferred proteins, DNA or RNA are then visualized by colorant staining (for example, silver staining of proteins), auto radiographic visually.

❖It is used in clinical chemistry to separate proteins by charge or size (IEF agarose, essentially size independent) and in biochemistry and molecular biology
are transferred to nitrocellulose filter.

❖This **method** can detect the presence or absence of bio molecule in a single run.

❖ Three types of blotting techniques are commonly used for visualizing particular macromolecules

Types of blotting techniques



Southern blotting

❖ It is used to detect DNA/ Identification of a specific DNA sequence using a specific DNA probe

Northern blotting

❖It is used to detect RNA/Identification of a specific RNA sequence using a specific RNA probe.

Western blotting

It is used to detect protein /Identification of a specific protein using specific antibodies

Southern blotting

It was developed by Edward M. Southern (1975).

Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules.

This technique is based on the principle of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization.

The Southern blot is used to detect the presence of a particular piece of DNA in a sample.

The DNA detected can be a single gene, or it can be part of a larger piece of DNA such as a viral genome.

The key to this method is hybridization.
Hybridization-process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target patient DNA.

Steps of southern blot

DNA is extracted from the cell.
Extracted DNA is digested by restriction enzyme into smaller fragments called restriction fragments.

These restriction fragments are then subjected to agarose gel electrophoresis.

These fragments will be separated on the gel according to their molecular weight. Smaller fragments migrate faster.

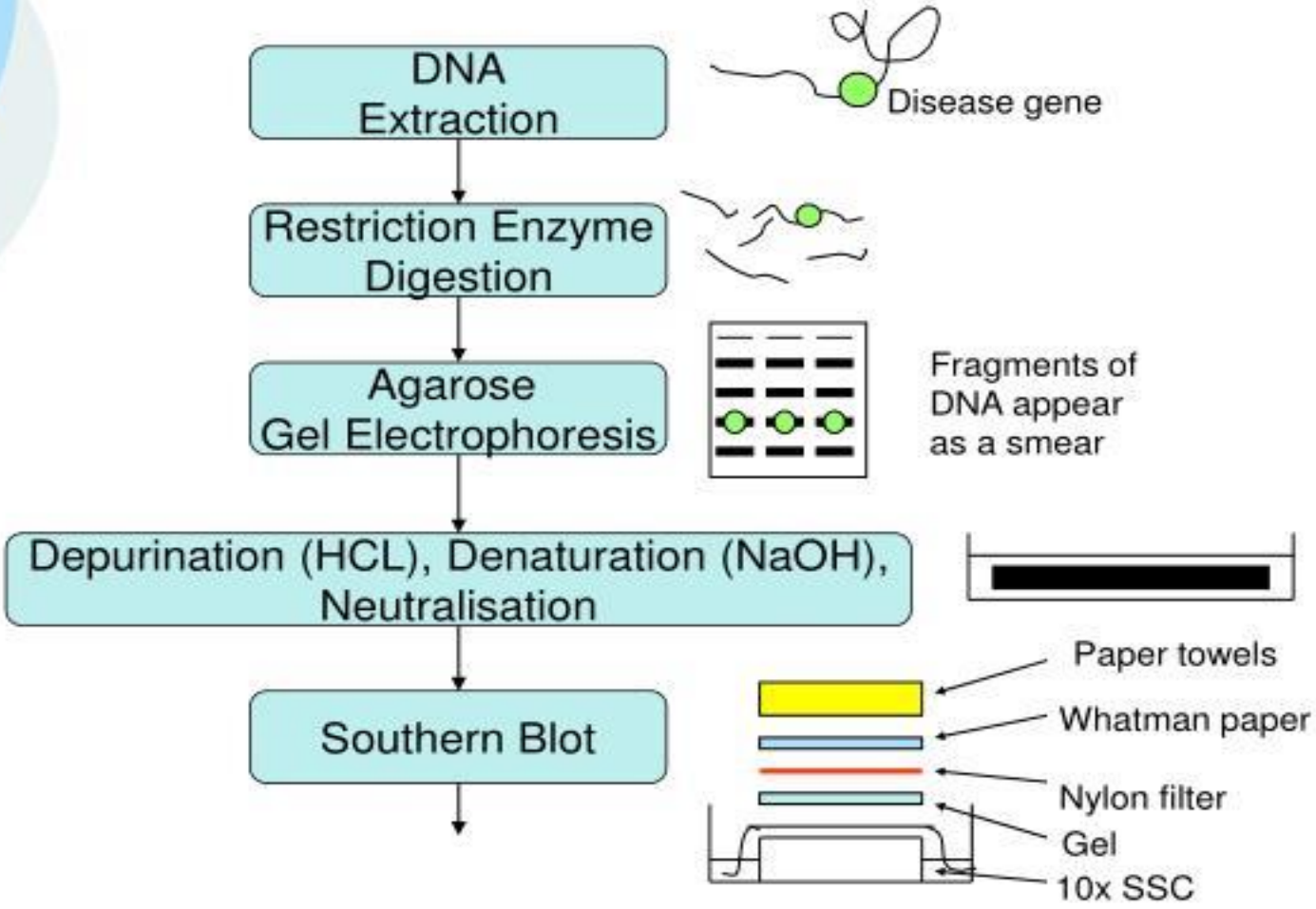
After electrophoresis, the gel is blotted on nitrocellulose membrane.

During blotting, the DNA fragments will be transferred by capillarity from the gel to the membrane.

Special probes are then applied to the membrane where they will combine with the complementary fragments.

These probes are labeled by radioactive isotope which could be easily detected by X-ray film as dark spots on the membrane.

Steps in Southern Blotting



DNA Purification

Isolate the DNA in question from the rest of the cellular material in the nucleus.

Incubate specimen with detergent to promote cell lysis.

Lysis frees cellular proteins and DNA.

Proteins are enzymatically degraded by incubation with proteinase.

Organic or non-inorganic extraction removes

DNA Fragmentation

Cut the DNA into different sized pieces.

Use restriction endonucleases (RE)
Nucleases hydrolyze the bonds that connect bases within the strand, resulting in cleavage of the strand.

They cleave the double stranded nucleic acid only at specific points In vivo, they are involved in DNA metabolism and repair or in bacterial host defence.

Gel Electrophoresis

Sorts the DNA pieces by size

Gels are solid with microscopic pores

Agarose or polyacrilamide

Gel is soaked in a buffer which controls the size of the pores

Standards should also be run

DENATURATION

The double stranded DNA is converted to single stranded DNA by alkali treatment method .

Blotting by Capillary action Transfer the DNA from the gel to a solid support.

The blot is usually done on a sheet of nitrocellulose paper or nylon.

DNA is then neutralized with NaCl to prevent re-hybridization before adding the probe.

Transferred by either electrophoresis or capillary blotting.

Hybridization

The labeled probe is added to the blocked membrane in buffer and incubated for several hours to allow the probe molecules to find their targets.

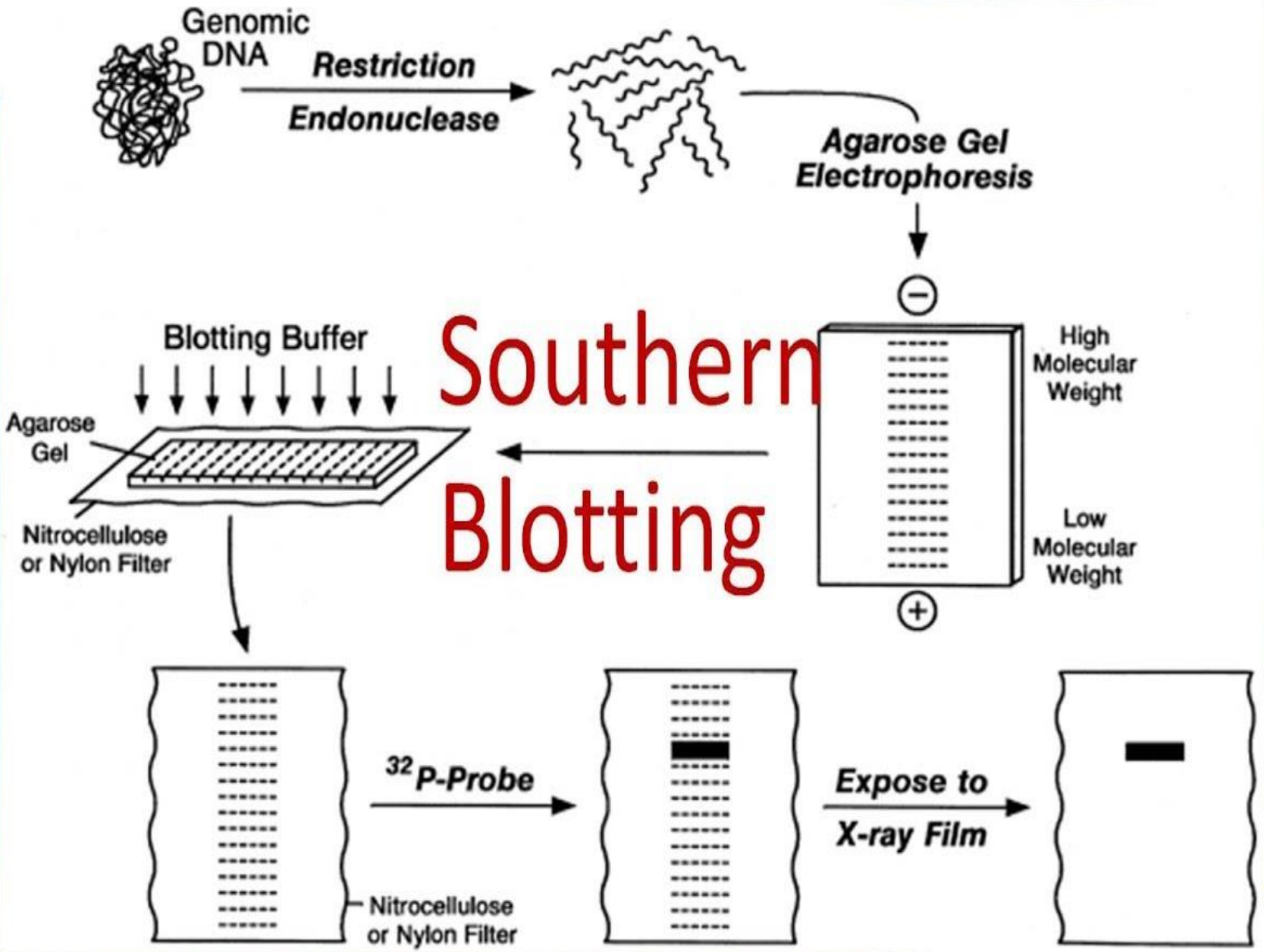
Steps for hybridization

1. The mixture of molecules is separated.
2. The molecules are immobilized on a matrix.
3. The probe is added to the matrix to bind to the molecules.
4. Any unbound probes are then removed.
5. The place where the probe is connected corresponds to the location of the immobilized target molecule.

Washing

Excess probe will have bound non-specifically to the membrane despite the blocking reagents.

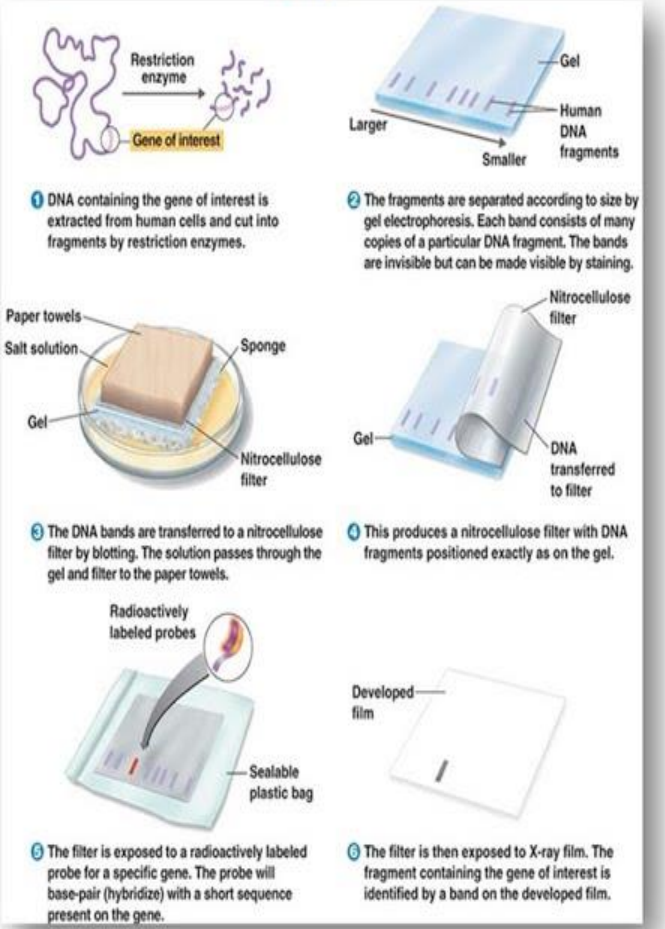
Blot is incubated with wash buffers containing NaCl and detergent to wash away excess probe and reduce background.


Genomic DNA
Restriction Endonuclease
Agarose Gel Electrophoresis
Southern Blotting
Blotting Buffer
Agarose Gel
Nitrocellulose or Nylon Filter
High Molecular Weight
Low Molecular Weight
32P-Probe
Nitrocellulose or Nylon Filter
Expose to X-ray Film

Southern Blotting

Principle, Procedure & Application

1. Extract and purify DNA from cells
2. DNA is restricted with enzymes
3. Separated by electrophoresis
4. Denature DNA
5. Transfer to nitrocellulose paper (Blotting)
6. Add labeled probe for hybridization to take place
7. Wash off unbound probe
8. Autoradiograph



www.biologyexams4u.com

SUMMARY OF PROCEDURE

1. Extract and purify DNA from cells

2. DNA is restricted with enzymes

3. Sort by electrophoresis

4. Denature DNA

5. Transfer to nitrocellulose paper
6. Block with excess DNA
7. Wash off unbound probe
8. Autoradiograph

Western blotting

Western blotting, is a widely used analytical technique in molecular biology and immunogenetics to detect specific proteins in a sample of tissue homogenate or extract.

Western blot

It consists of two steps

1- SDS PAGE

2- Immunoblot

SDS PAGE

First, the protein sample is subjected to electrophoresis on SDS polyacrylamide gel.

SDS sodium dodecyl sulfate unfolds the complex structure of the proteins and loads them with negative charges.

On the gel, the proteins will move toward the positive electrode and separated according to their molecular weight.

Smaller proteins migrate faster.

Immunoblot

The gel is then blotted on nitrocellulose membrane using electrophoresis inside immunoblot apparatus.

Note that inside the immunoblot apparatus, the gel must be placed toward the negative electrode and the membrane is placed toward the positive electrode.

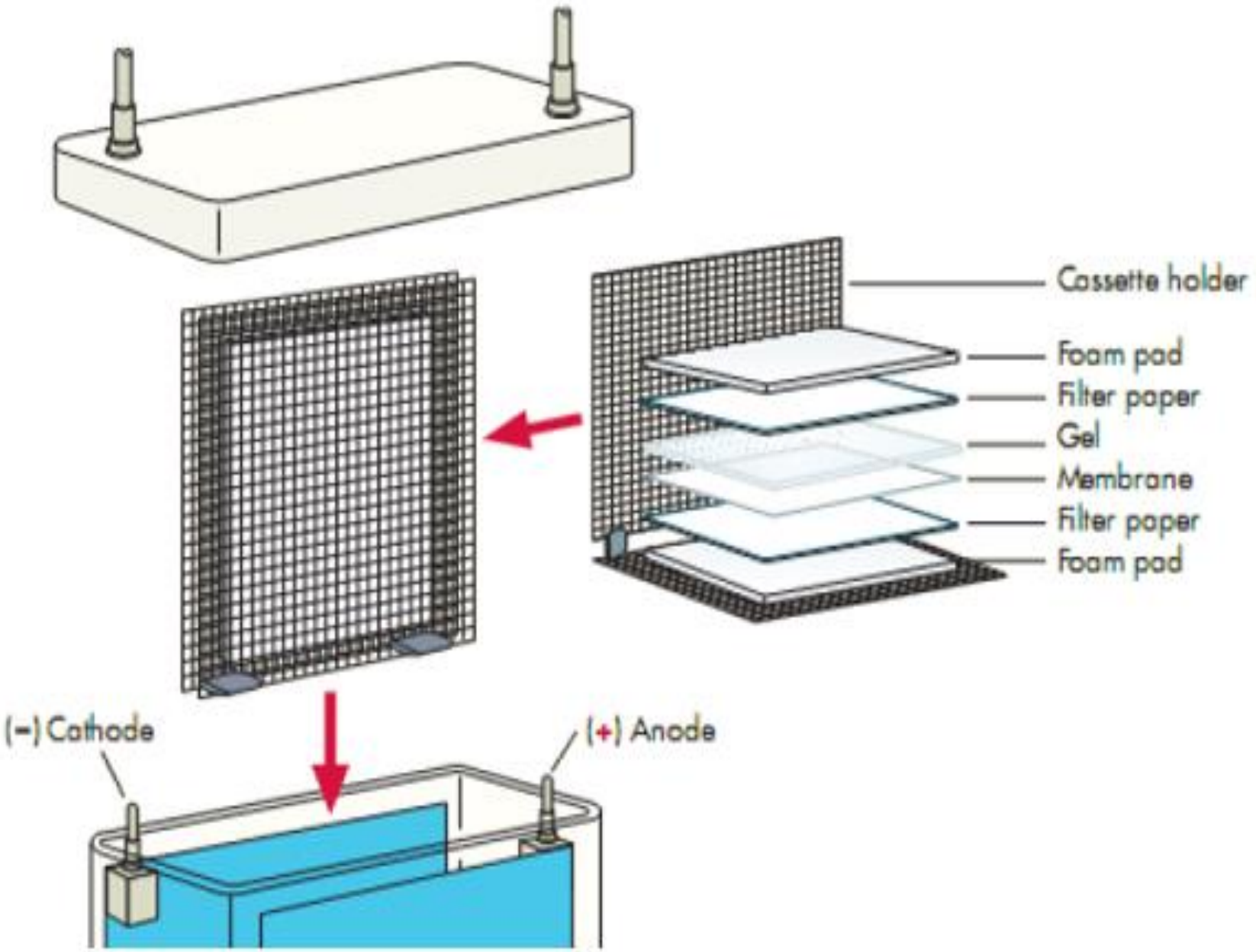
Inside the immunoblot apparatus, proteins on the gel will be transferred to the membrane.

After blotting, the membrane is blocked by adding serum albumin which prevents nonspecific absorption of antibodies that will be added to the membrane.

Then, the membrane is mixed with primary antibody against the protein of interest.

After washing the membrane, it is mixed with secondary antibody against the primary antibody.

Note that secondary antibody is labelled with enzyme.


Cassette holder
Foam pad
Filter paper
Gel
Membrane
Filter paper
Foam pad
(−) Cathode
(+) Anode

After washing the membrane, a substrate for the enzyme is added to the membrane.

The enzyme acts on the substrate producing color at the site of protein of interest.

Applications of blotting techniques

Diagnosis of infectious diseases like tuberculosis.

Diagnosis of tumors like leukemia and lymphoma.

Diagnosis of genetic diseases.
In forensic medicine like paternity testing.


THANK YOU