FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

What is blotting

a Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis. The Southern blot is used for transferring DNA, the Northern blot for RNA and the western blot for PROTEIN.

Gel Electrophoresis

- The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point molecular weight, electric charge, or a combination of these factors.
- The principle involved is the difference in the ELECTROPHORETIC MOBILITIES of different proteins.

Processing

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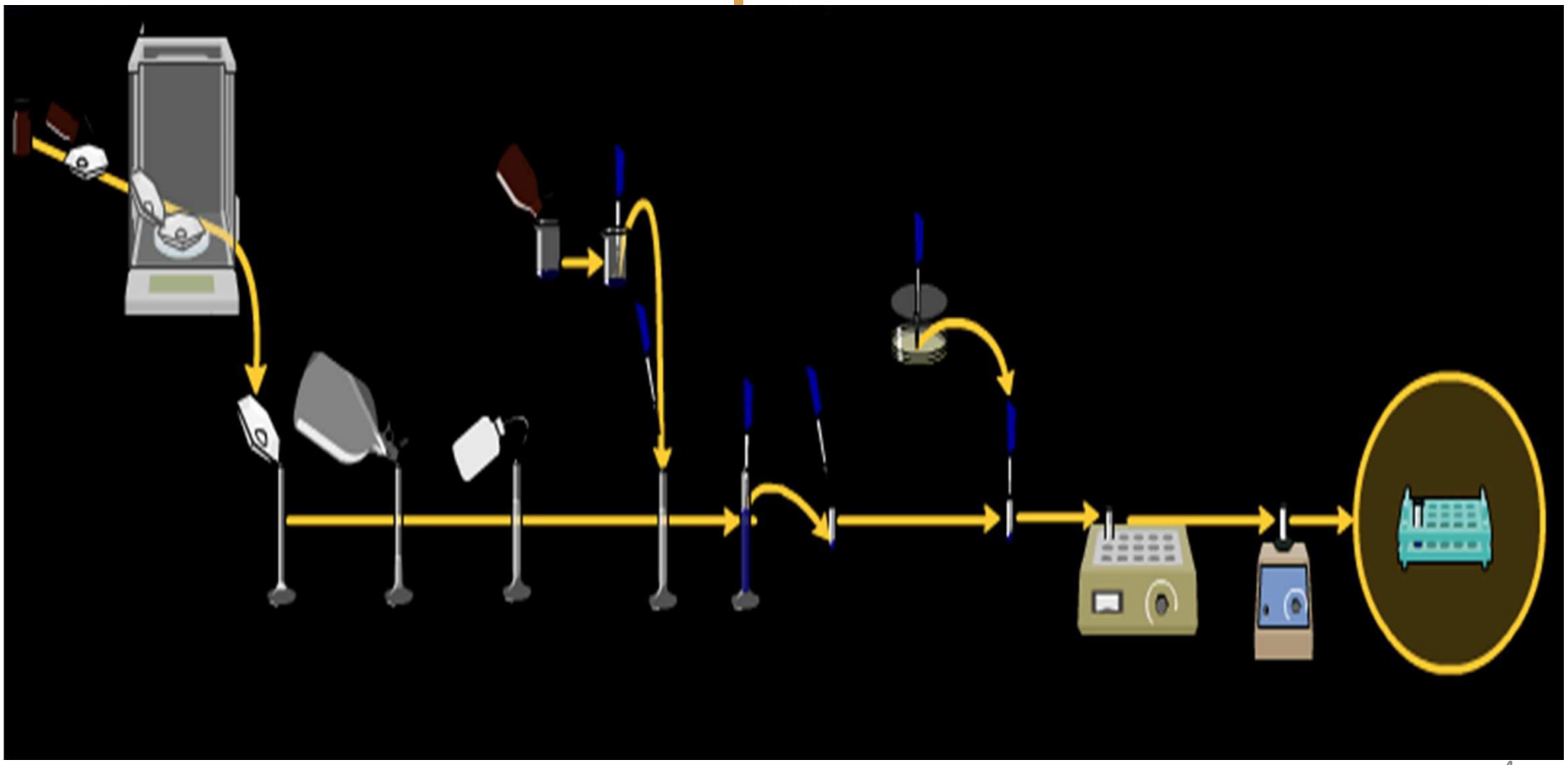


Transferring

a In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or polyvinylidene difluoride (PVDF). The membrane is placed on top of the gel, and a stack of filter papers placed on top of that. The entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it.

Another method for transferring the proteins is called electro blotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane.

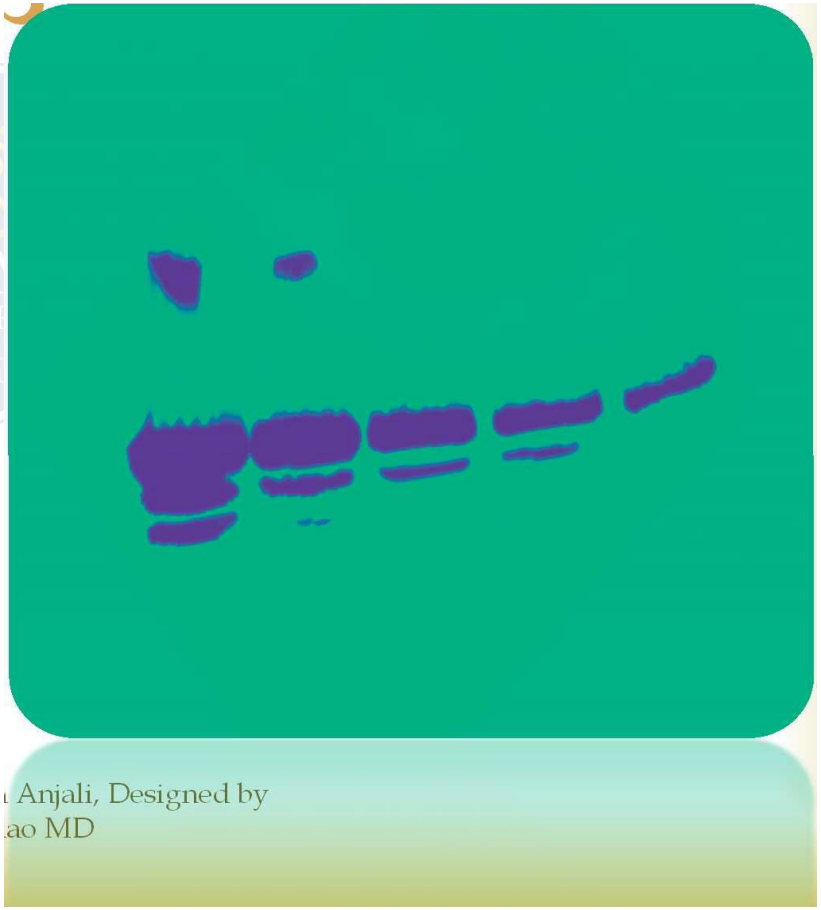
Blocking

- The membrane has the ability to bind to proteins in in this case both the target and antibodies are proteins and so there could be some unwanted binding.
  - Blocking of non-specific binding is achieved by placing the membrane in a dilute solution of protein - typically Bovine serum albumin(BSA) with a minute percentage of detergent such as Tween 20.
  - The protein in the dilute solution attaches to the membrane in all places where the target proteins have not attached.

  Thus, when the antibody is added, there is no room on the membrane for it to attach other than on the binding sites of the specific target protein.

Detection

- During the detection process, the membrane is "probed" for the protein of interest with a modified antibody which is linked to a reporter enzyme, which when exposed to an appropriate substrate drives a colorimetric reaction and produces a color.



Analysis

- After the unbound probes are washed away, the western blot is ready for detection of the probes that are labeled and bound to the protein of interest.
- Size approximations are taken by comparing the stained bands to that of the marker loaded during electrophoresis.
- The process is repeated for a structural protein, such as actin or tubulin that should not change between samples.

Advantages

While ELISA being a non specific test, Western blotting is a more specific test for detection of HIV.

It can detect one protein in a mixture of proteins while giving information about the size of the protein and so is more specific.

Western blot test is referred to as the 'Gold Standard'

It also tells you how much protein has accumulated in cells.

Western Blot in Clinical Medicine

- The confirmatory HIV test employs a Western blot to detect anti-HIV antibody in a human serum sample. Proteins from known HIV-infected cells are separated and blotted on a membrane then, the serum to be tested is applied in the primary antibody incubation step; free antibody is washed away, and a secondary anti-human antibody linked to an enzyme signal is added. The stained bands then indicate the proteins to which the patient's serum contains antibody.
- A Western blot is also used as the definitive test for Bovine spongiform encephalopathy (BSE, commonly referred to as 'mad cow disease').
- Some forms of Lyme disease testing employ Western blotting.

Western Blot a Confirmatory test in HIV Infection

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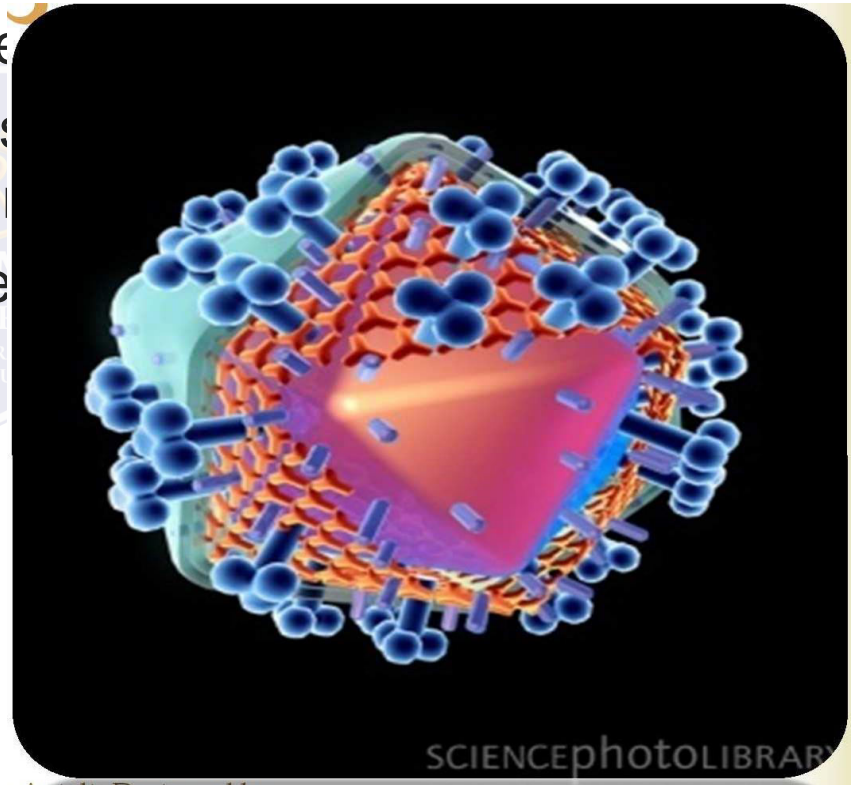

Anjali, Designed by
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