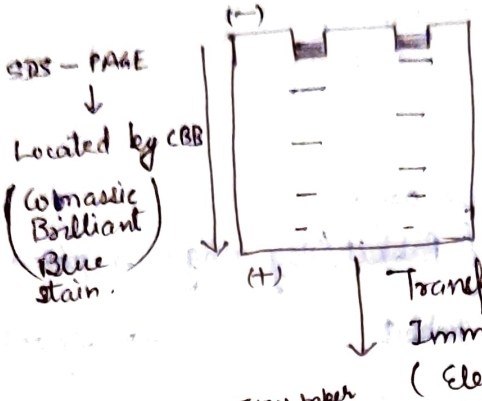
# -: WESTERN BLOTTING :-

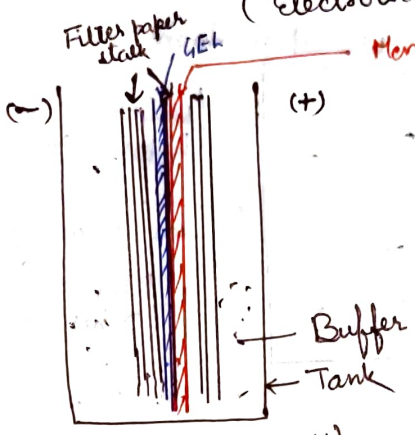
By W. Neal Burnett (1980)

Starting material : Impure preparation
[Cellular / Tissue lysate]



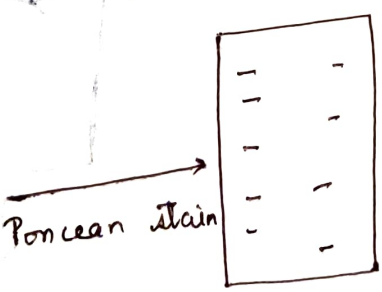

Transferred
Immobilization
(Electrotransfer)

Western transfer / blotting



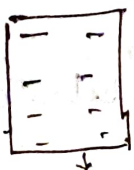

① Filter paper (Whatman 3M)
② 3 layer of filter paper on both side of gel.
③ Membrane (Nitrocellulose / PVDF) on the side and 3 filter paper.

a) Wet technique – Put in buffer.
b) Semi dry – Filter paper on both side wet in buffer.




Whole membrane is dipped in Poncean stain to see prot
transfer whether it has occured or not.

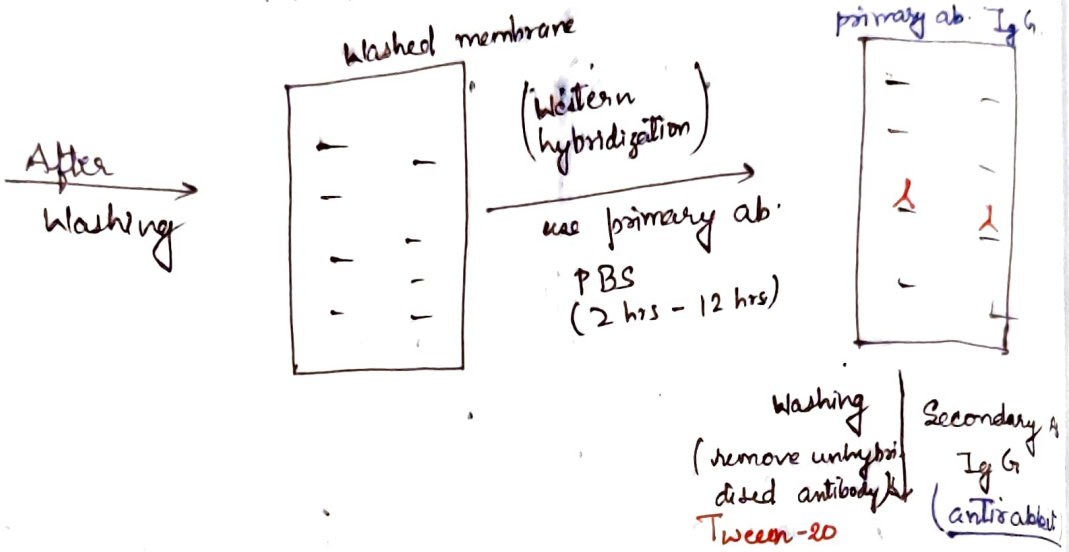
↓ Washed membrane by PBS (phosphate buffer saline 100 mM NaCl) so that st
comes out.

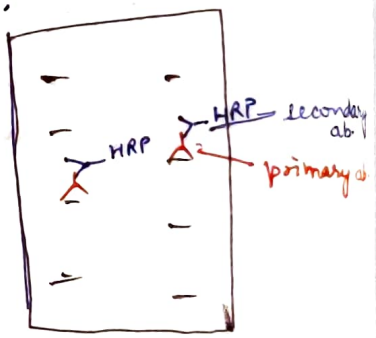


↓ Blocking (covering of region non-specific)
(Non fat milk) or Casein protein
Bovine Serum Albumin (BSA)

↓
They don't bind with protein rather fo
empty regions of membrane only. Since
are heterologous to proteins.

NOTE: Antigen of mouse origin
Antibody of Rat / Rabbit / Dog origin → primary antibody
Antibody of Dog Rabbit → secondary antibody



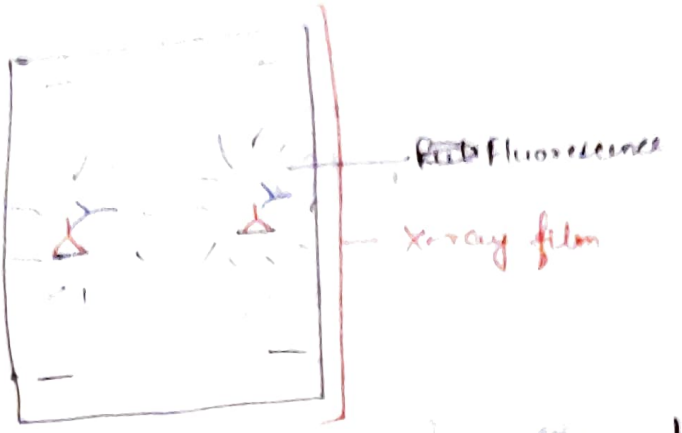

↓ Detection.

Secondary ab., conjugated with enzyme HRP (Horse Radish Peroxidase) or AP (Alkaline Phosphatase)

↓ submerged in medium having substrate.

a) For HRP

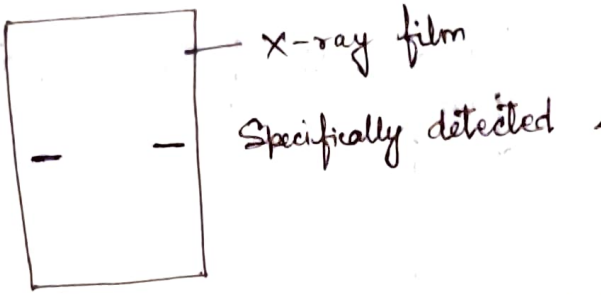
$H_2O_2 \xrightarrow{HRP} H_2O + O_2$ + oxidised luminol → (Fluorescence)




Amount of light get captured in X-ray film in dark room.

↓ ECL → Enhanced Chemiluminiscence. It is a detection technique eg. Luminol.

X-ray film developed and fixed.




b) DAB (Diaminobenzedine) → Non ECL method.

↓

Chromogenic substance [which produces colour eg. BCIP (AP) DAB (HRP)]

$DAB + O_2 \xrightarrow{HRP}$ Oxidised DAB

↓

Brown ppt in membrane.

## 2. If enzyme is AP (Alkaline phosphatase) –

① BCIP (5-Bromo 4-chloro 3-indolyl phosphate)

↓

chromogenic substance.

– Here phosphate moiety removed so (5-Bromo-4-Chloro 3-indolyl (Dimer)) is formed which is blue coloured and very light.

↓

Use NBT (Nitro Blue Tetra-azonium salt) To remove blue colour. (more intense)

Complex formed e/a Formazen complex.

↓

Intense blue.