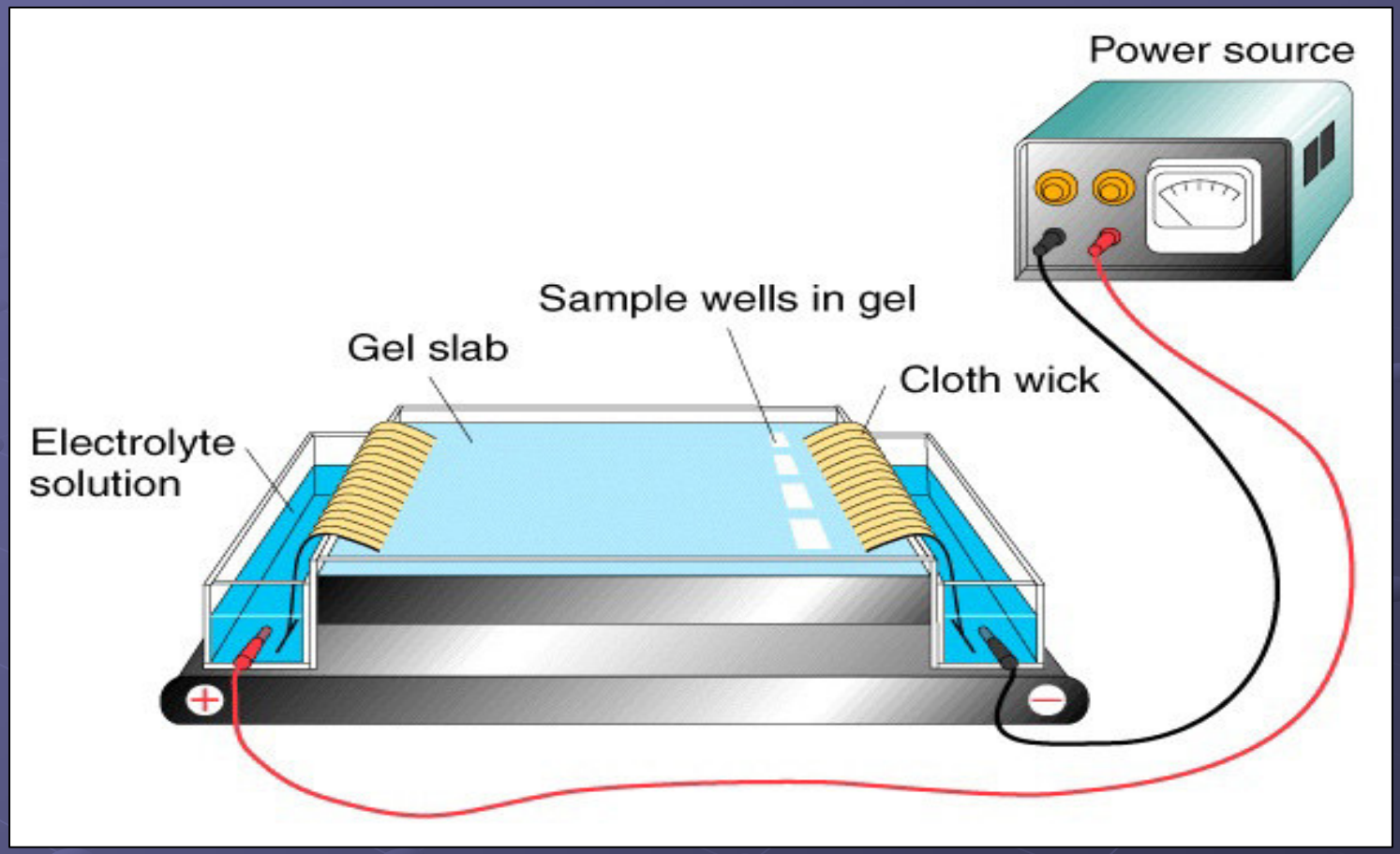
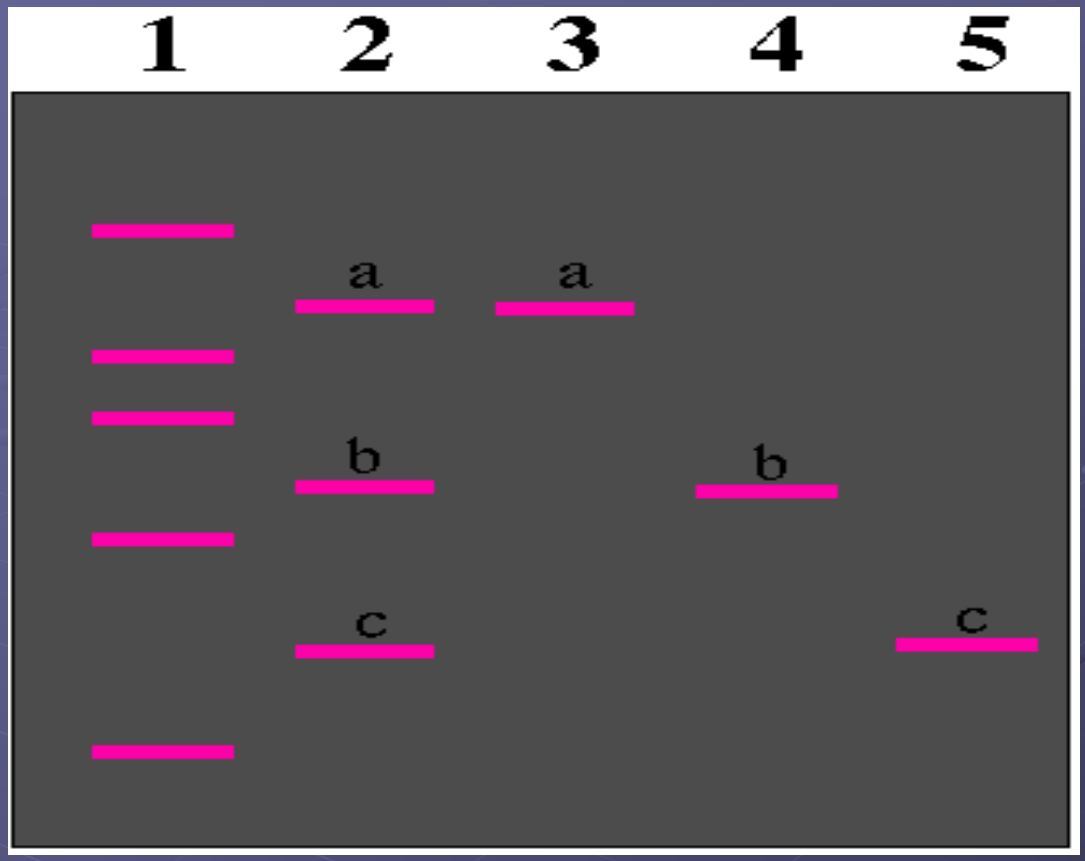
# Electrophoresis and transfer

# Electrophoresis

- **Cation** = positively charged ion, it moves toward the cathode (-)
- **Anion** = negatively charged ion, it moves toward the anode (+)
- **Amphoteric substance** = can have a positive/negative/zero charge, it depends on conditions

- Principle:
- Some substances have different net charges and can be separated into several fractions in external electric field.
- But velocity of a particle also depends on the:
- **size, shape of the particle and given applied voltage**

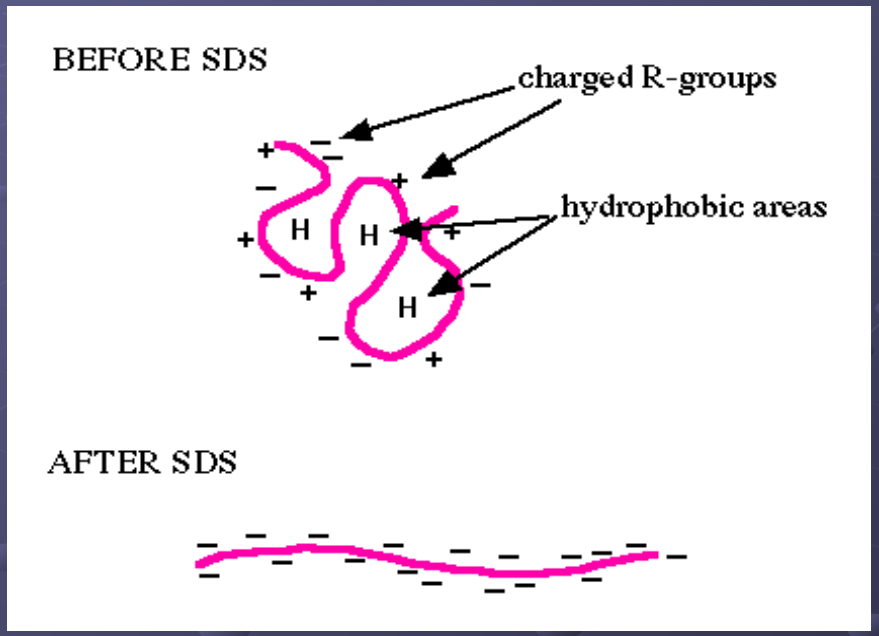

Power source
Sample wells in gel
Gel slab
Cloth wick
Electrolyte solution
+
−




The actual bands are equal in size, but the proteins within each band are of different sizes.

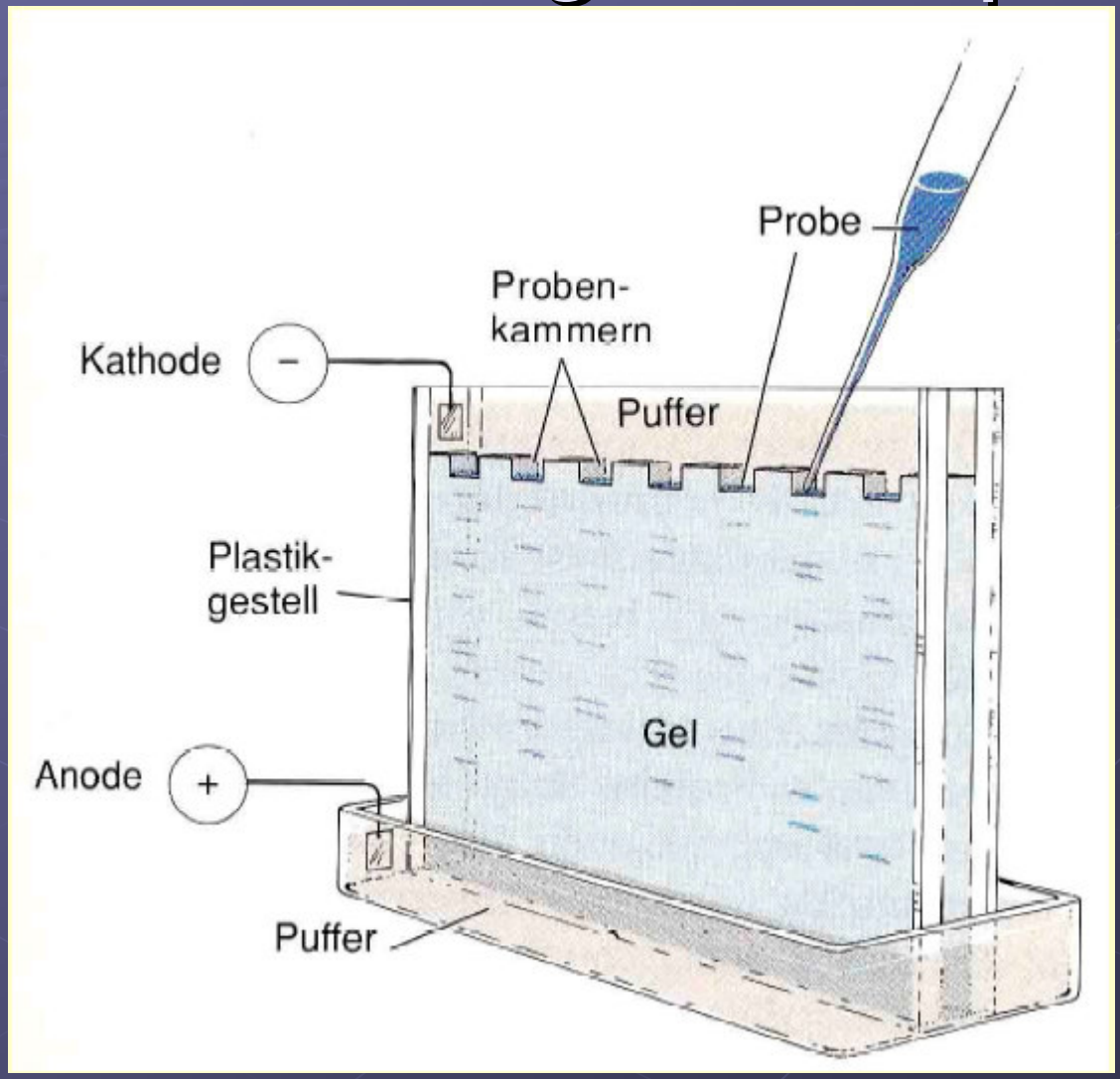
# SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmli 1970)

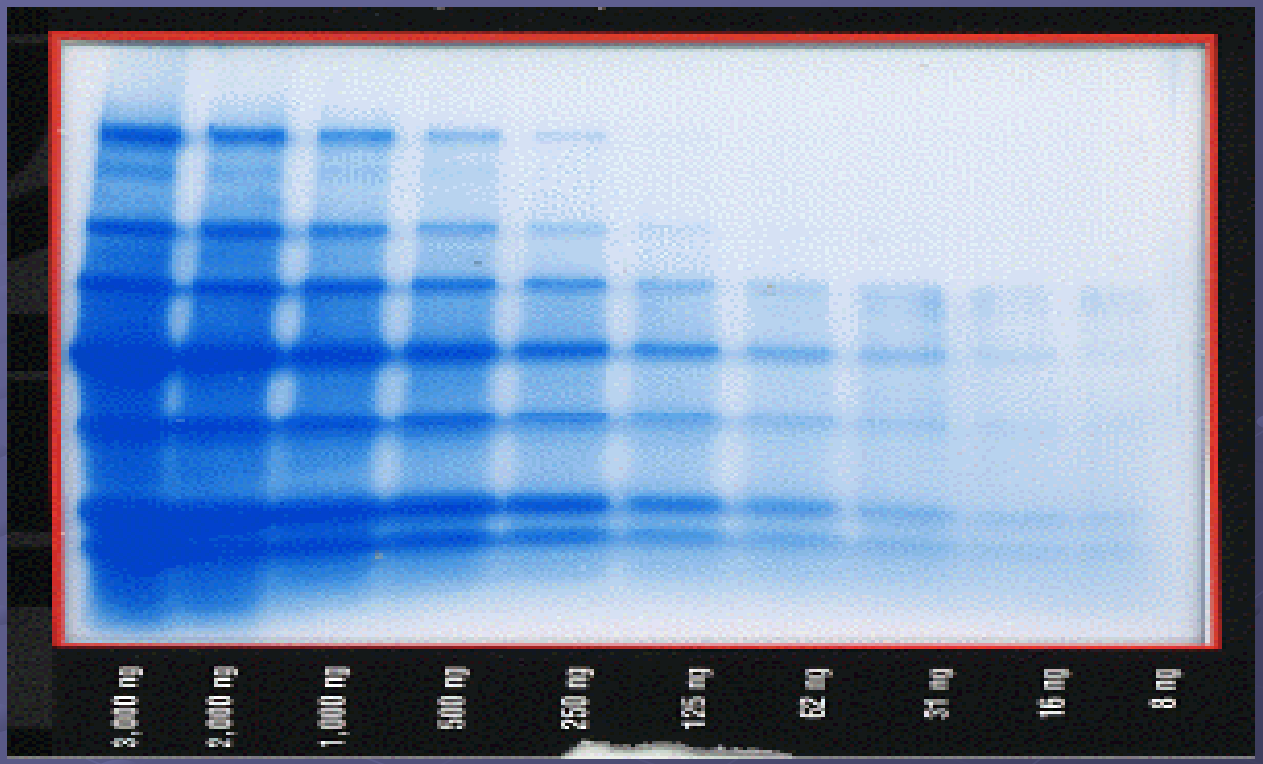
**SDS** (sodium dodecyl sulfate) is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge




•Therefore, if a cell is incubated with SDS, the membranes will be dissolved, all the proteins will be solubalized by the detergent and all the proteins will be covered with many negative charges.

# Vertical gel set up






**Protein gel (SDS-PAGE) that has been stained with Coomassie Blue.**


- Serum proteins are separated into 6 groups:
  - Albumin
  - α1 - globulins
  - α2 - globulins
  - β1 - globulins
  - β2 - globulins
  - γ - globulins

# Terminologies..

- The **Western blot** (alternatively, **protein immunoblot**) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract.
- A **Southern blot** is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.
- The **northern blot** is a technique used in molecular biology research to study gene expression by detection of RNA.
- **Southwestern blotting**, based along the lines of Southern blotting (which was created by Edwin Southern) and first described by B. Bowen and colleagues in 1980, is a lab technique which involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA).

# Western Blotting (WB)

WB is a protein detection technique that combines the separation power of SDS PAGE together with high recognition specificity of antibodies

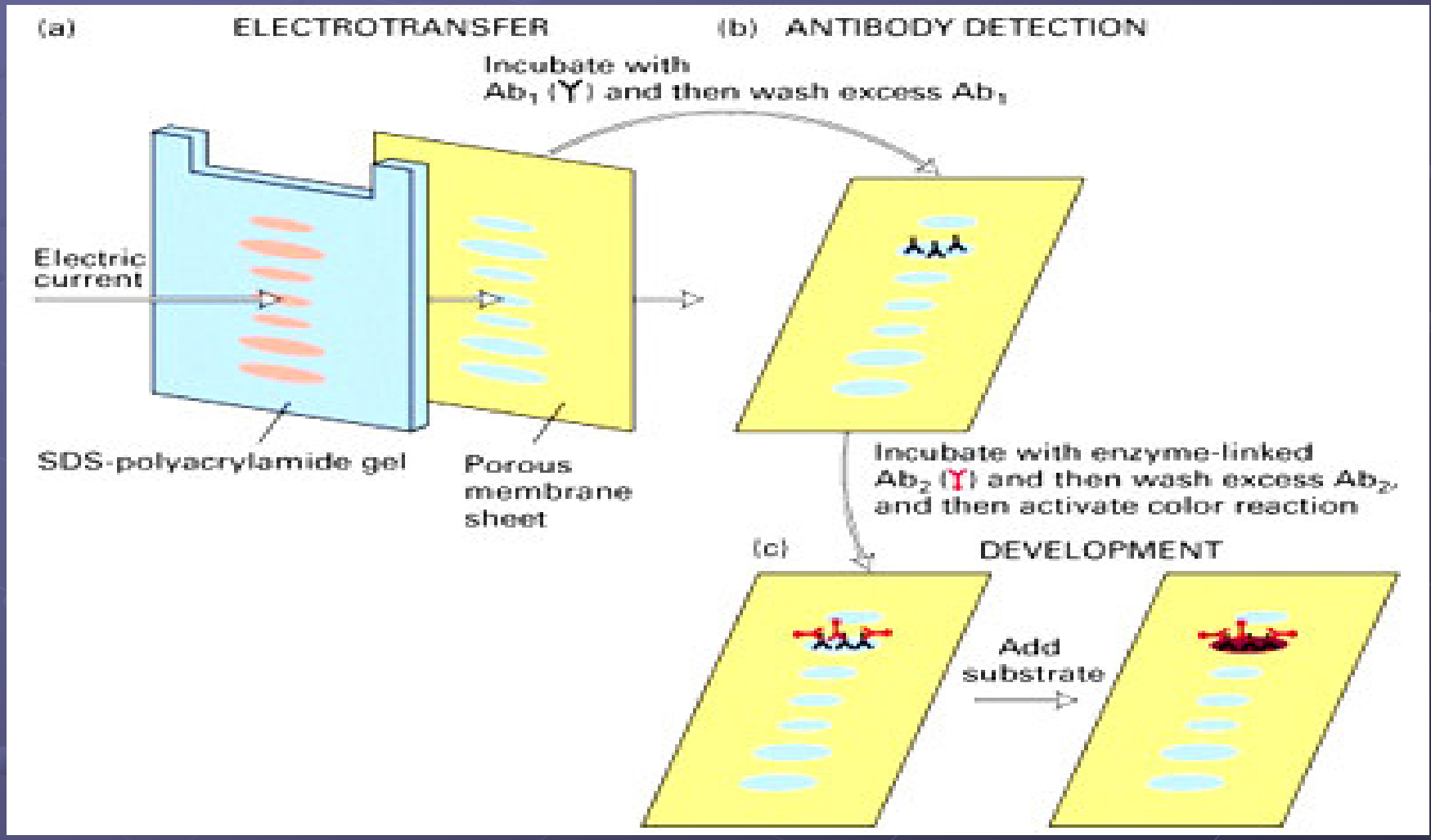
An antibody against the target protein could be purified from serum of animals (mice, rabbits, goats) immunized with this protein

Alternatively, if protein contains a commonly used tag or epitope, an antibody against the tag/epitope could be purchase from a commercial source (e.g. anti-6 His antibody)

# WB: 4 steps

1. Separation of proteins using SDS PAGE
2. Transfer of the proteins onto e.g. a nitrocellulose membrane (blotting)
3. Immune reactions
4. Visualization

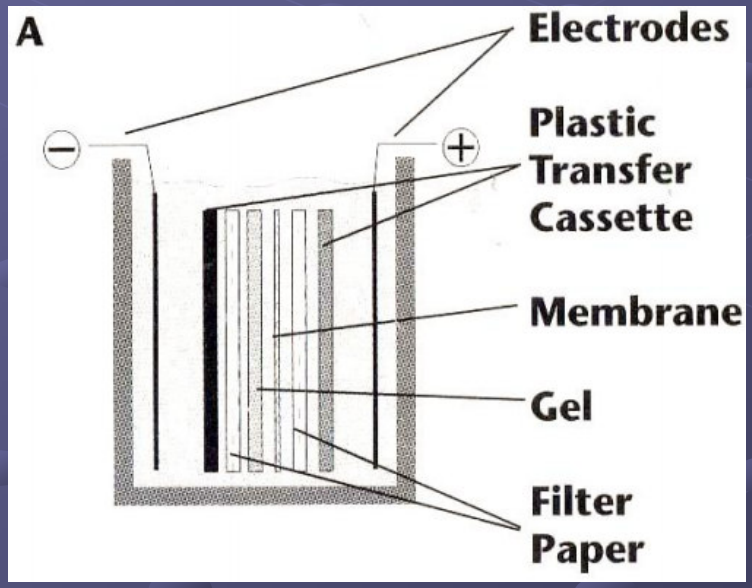
# The essence of Western-blot

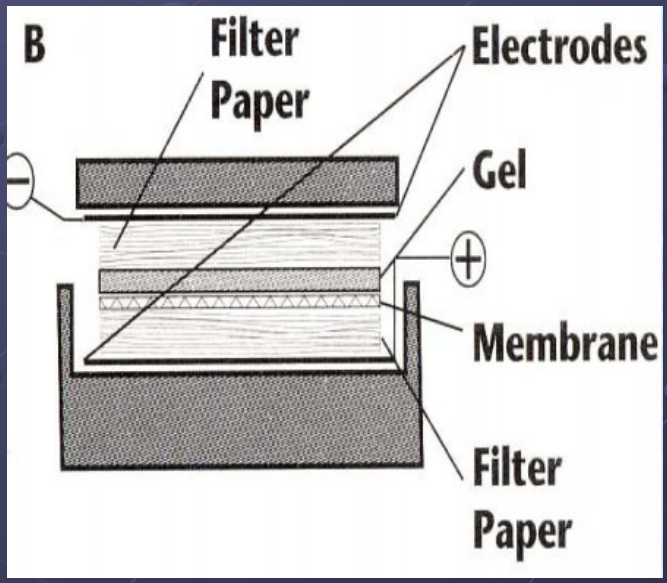


# Transfer

Wet

Semi-dry



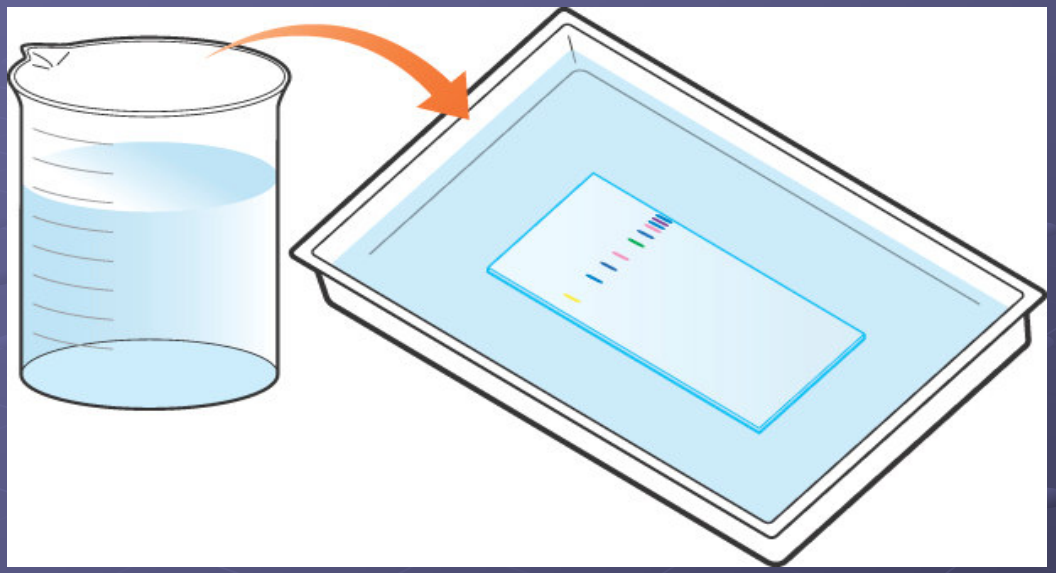
# Types of membranes

Nitrocellulose (NC)  high binding capacity, works well with both protein and DNA not need methanol to preparation.

Polyvinylidene difluoride (PVDF)  high capacity and stable, need methanol for preparation.

These both membranes bind proteins non-covalently.

# Blocking



5% non-fat milk or BSA with Tween 20: Prevents the primary antibody from binding randomly to the membrane

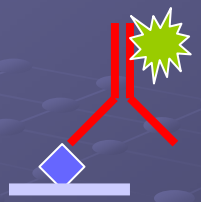
After blocking apply your first Ab at the specific concentration, learn how…? Wash carefully, apply secondary Ab HRB conjugated, eash carefully, detect your specific protein by detection reagent.


thank you

# Methods based on specific antigen and antibody binding

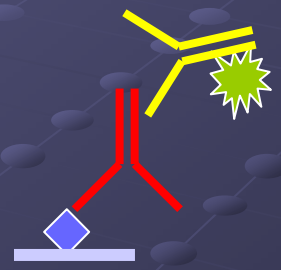
**Direct method**

We label the antigen or the antibody



**Indirect method**

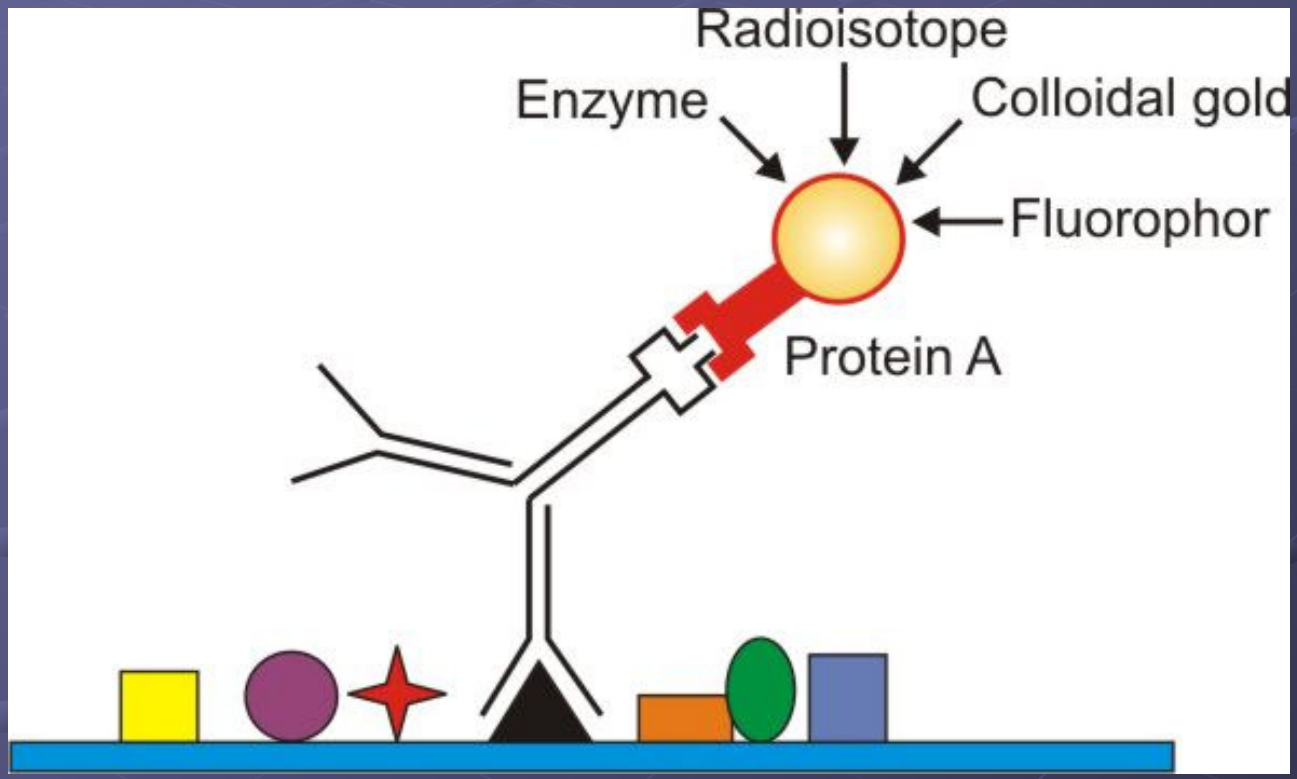
We detect the antigen-antibody binding with a labelled anti-immunglobulin antibody (e.g. goat anti-human IgG) that recognize the specifically reacting primary antibody mutatjuk ki. Method is mainly applied to detect antigen specific antibodies and for their. Increased specificity.



**Double antibody: „sandwich" method**

In this method we bind the antibody-molecules reacting specifically with the antigen to solid phase. The anchored antibody specifically binds the antigen, thus the antigen isolated from multicomponent solution. The antibody-antigen binding is the detected by another specifically reacting labelled antibody.

# WB, Steps 3-4: Detection



# Method for detection of western blot

## Most famous

1- colorimetric, by substrate that affected by atomic O resulted from H2O2 hydrolysis by HRB enzyme linked to secondary antibody and give colour

2- ECL, a reagents that affected by atomic O and give luminescence that filmed on X ray films in a dark room, more sensitive that colorimetric method

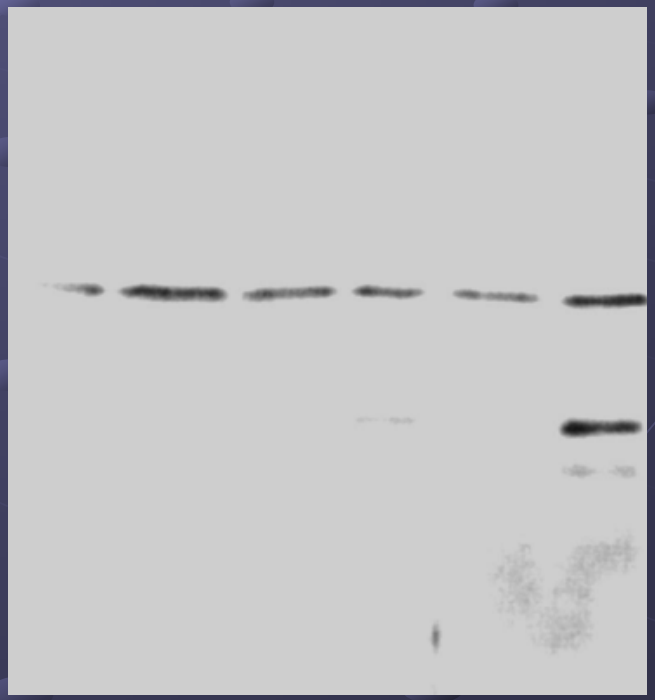
# Comparison between ECL and DAB detection methods

ECL is more sensitive 3-5 folds

Look carefully , is your protein found in coomassie blue stain?

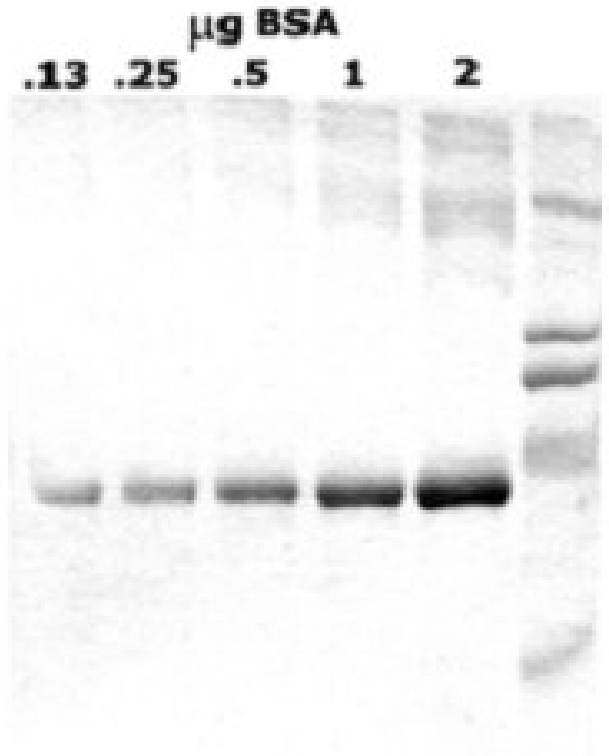
SDS page stained with coomassie blue

**The same but after Western blot**



# Quantifying Proteins

- subjective estimates
- scanning densitometry
- excise bands and count radioactivity



# Protein Detection

## General Proteins

- Coomassie blue
- silver stains
- fluorescence
- radioactivity

## Specific Proteins

- antibody/immunoblot
- enzyme activity
  - protease activity
  - redox reactions

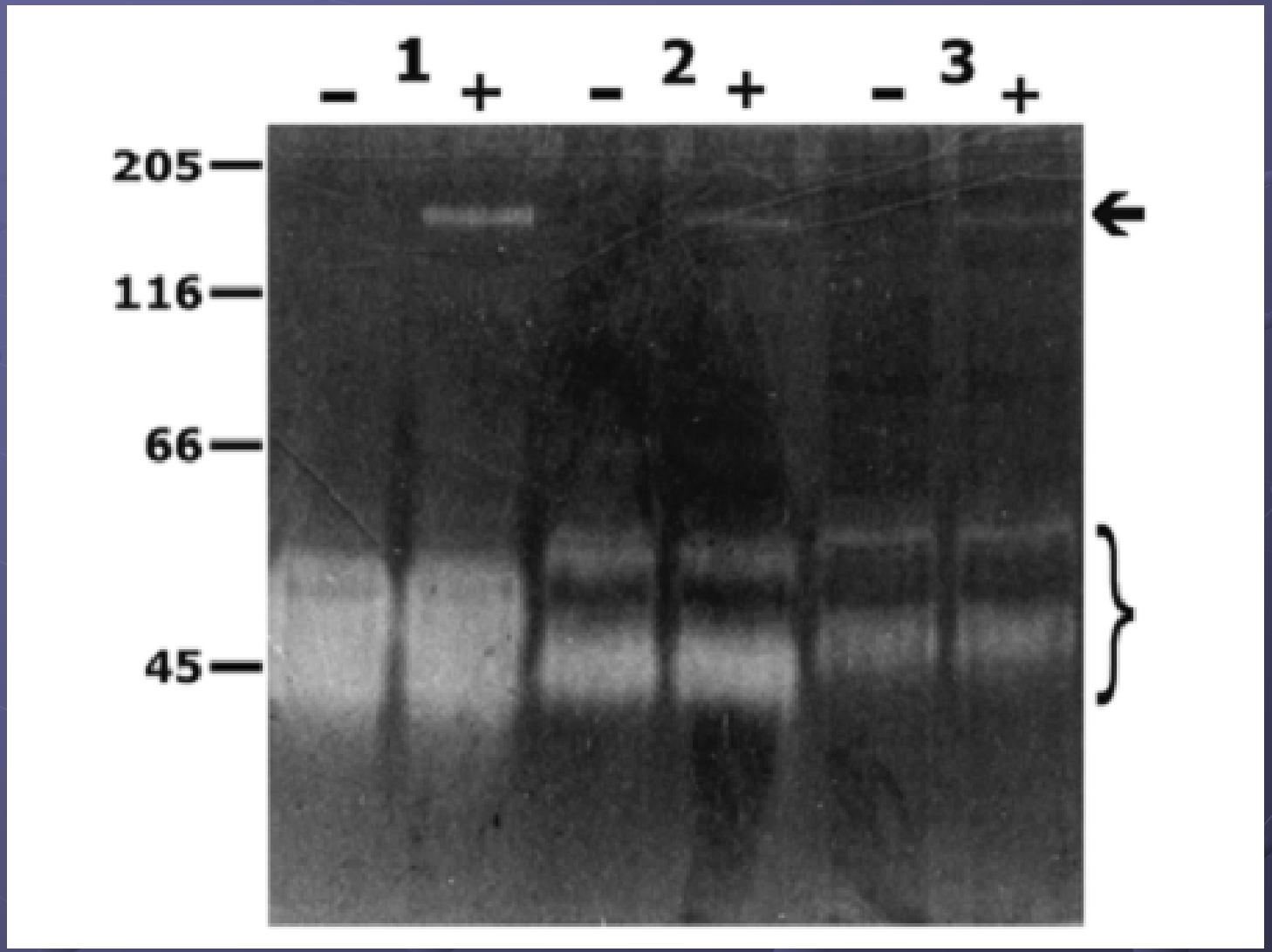
## Activity Gels

- carry out electrophoresis under native conditions
- or remove SDS following SDS-PAGE
  - some proteins refold
  - lower SDS and no heat
  - replace with non-ionic detergent

## Protease Activity

- co-polymerize PAG with protein substrate
- clear zones following incubation and staining

# Detection of enzyme activity by the colored substrate in SDS page



# MW estimation

Method 1:

Amino Acids approx 110 daltons

# residues x 110 dalton/residue = MW

Method 2:

Run SDS PAGE with known standards (MW markers)

Graph

Measure distance unknown protein travelled

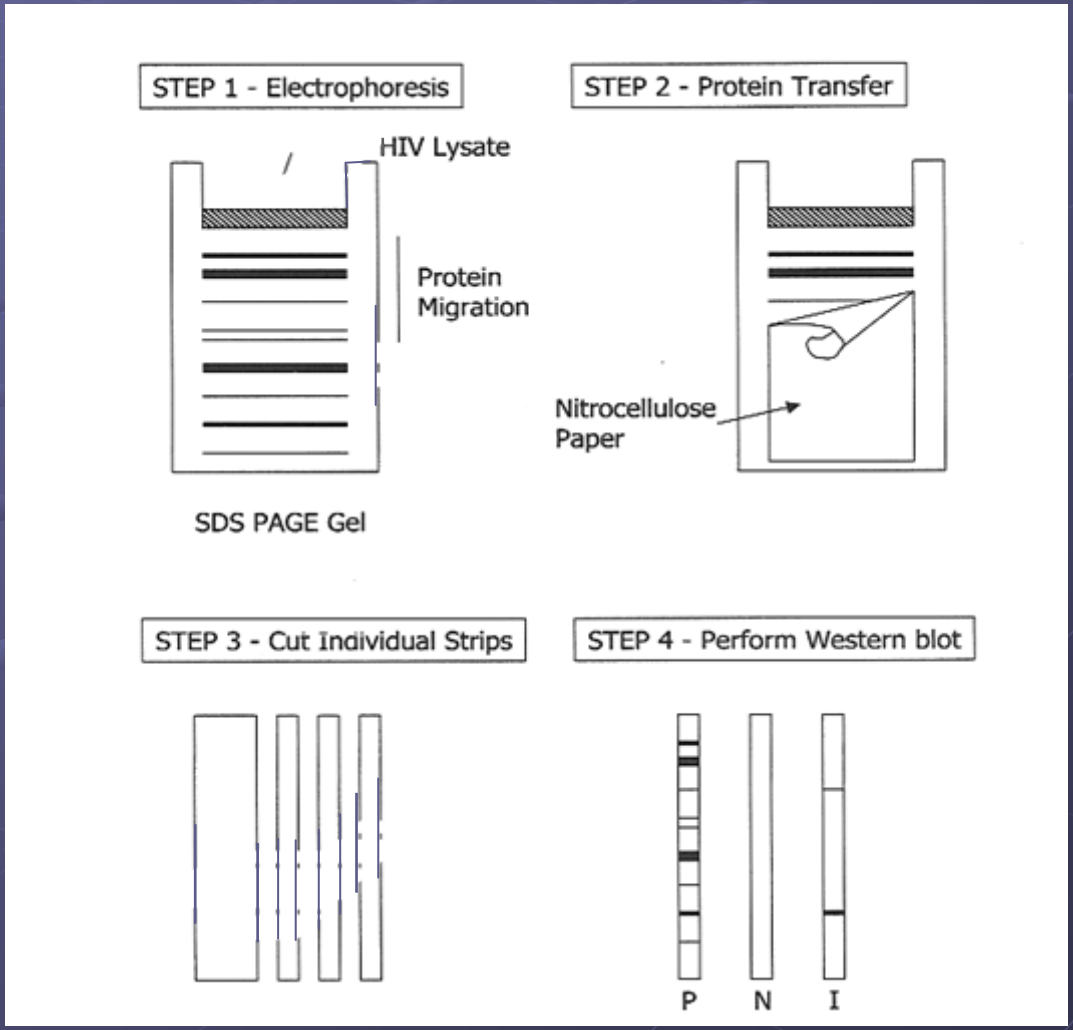
Compare on standard curve

# Western blot application

## HIV test

HIV lysate proteins are separated by size using gel electrophoresis

The membrane is cut into strips

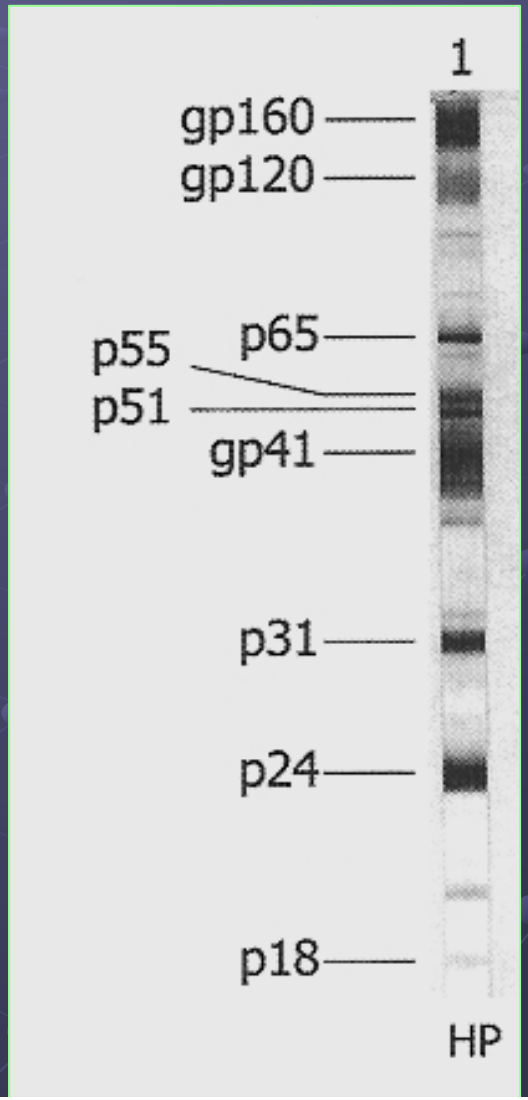



Proteins are transferred (blotted) onto the surface of a membrane

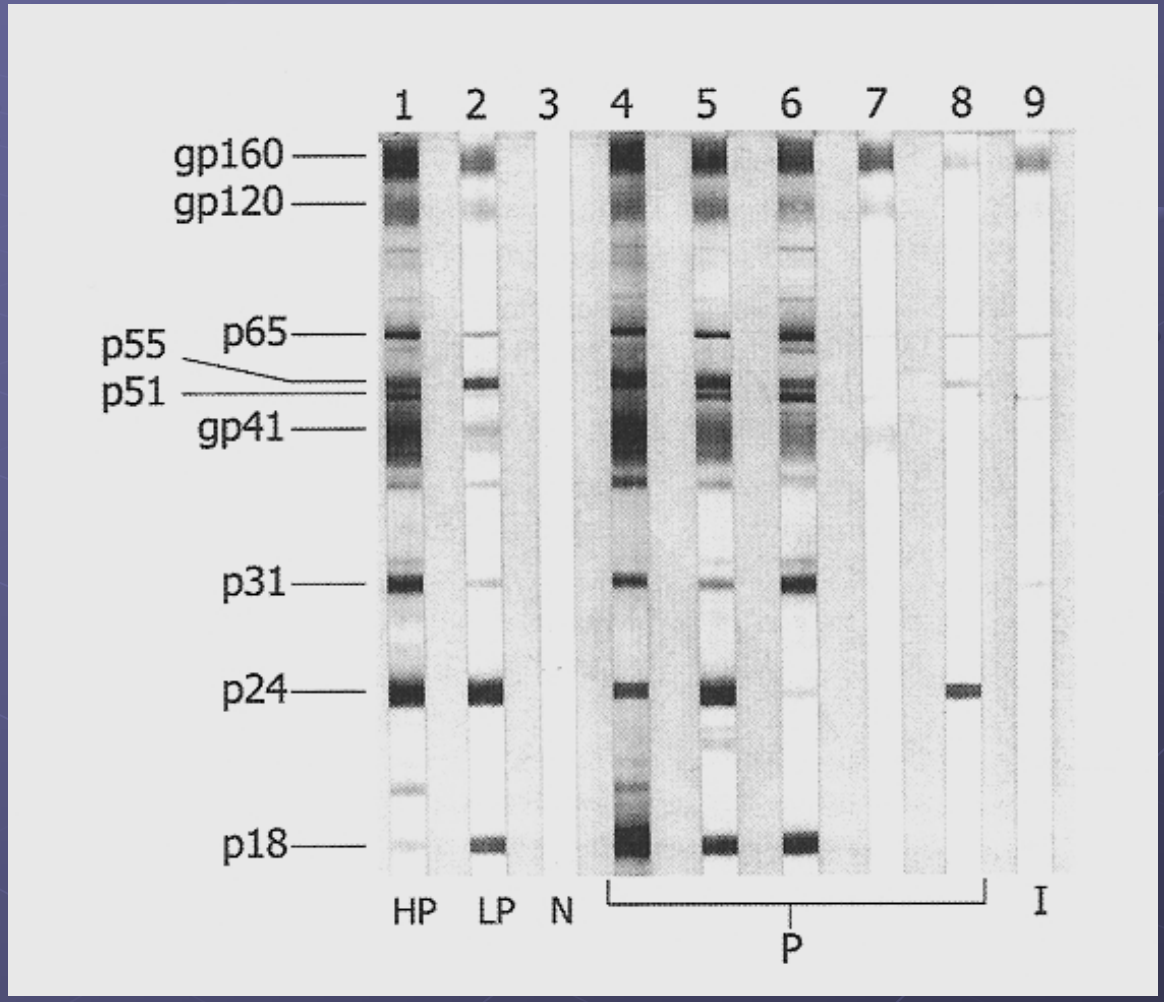
Strips are incubated with patient serum and antihuman IgG conjugated with an enzyme (and chromagen)

# HIV Western Blot Banding Pattern

| | |
|---|---|
| env | gp160 |
| | gp120 |
| | gp 41 |
| gag | p55 |
| | p18 |
| | p24 |
| pol | p65 |
| | p51 |
| | p31 |



# Western Blot Banding



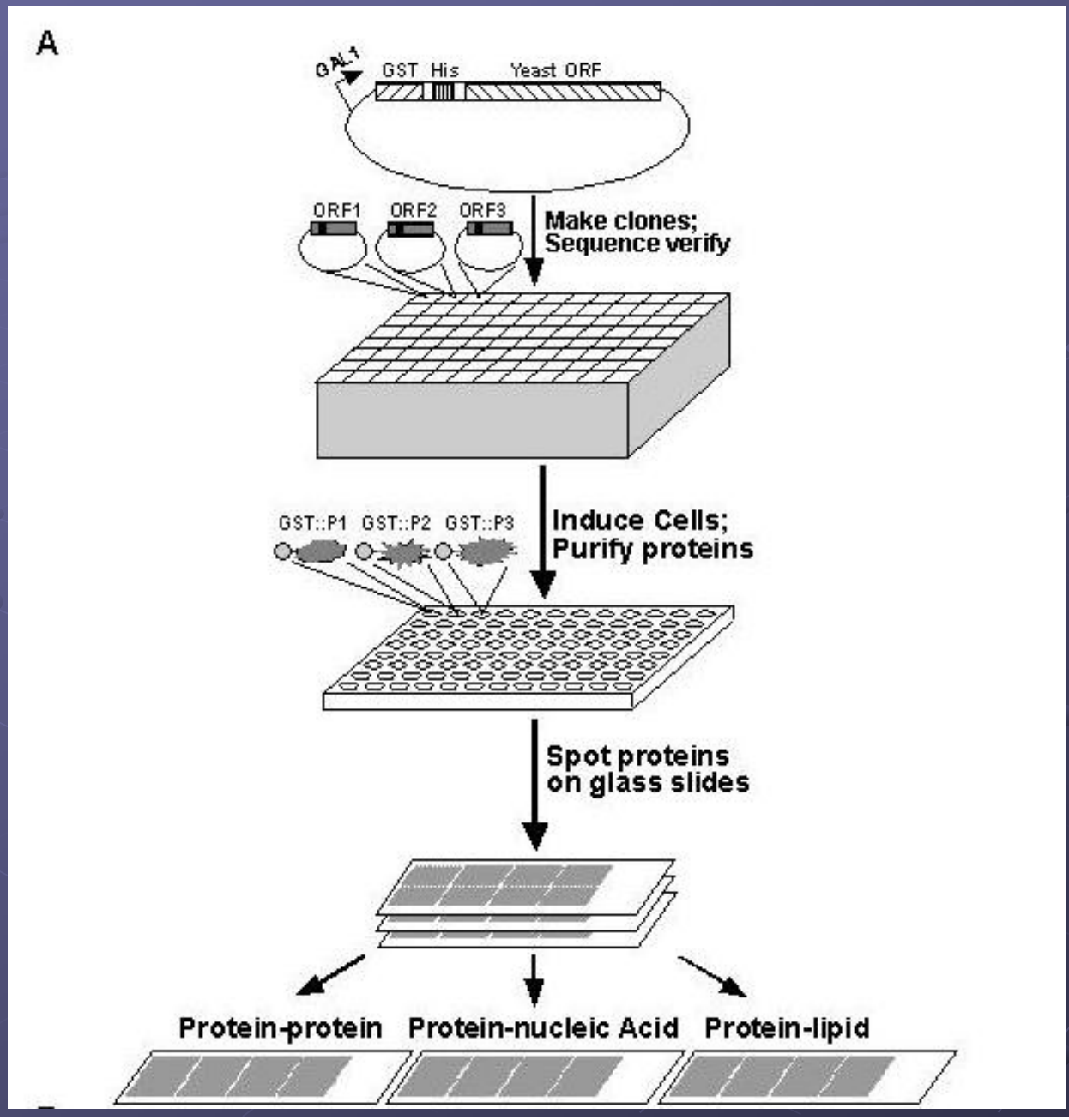
# Interpretation of Results (General Consensus)

**Negative:** No bands present

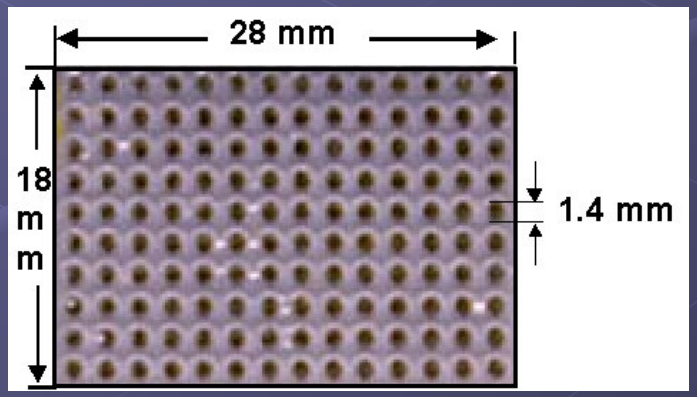
**Positive:** 2 ENV band present (WHO Guidelines)

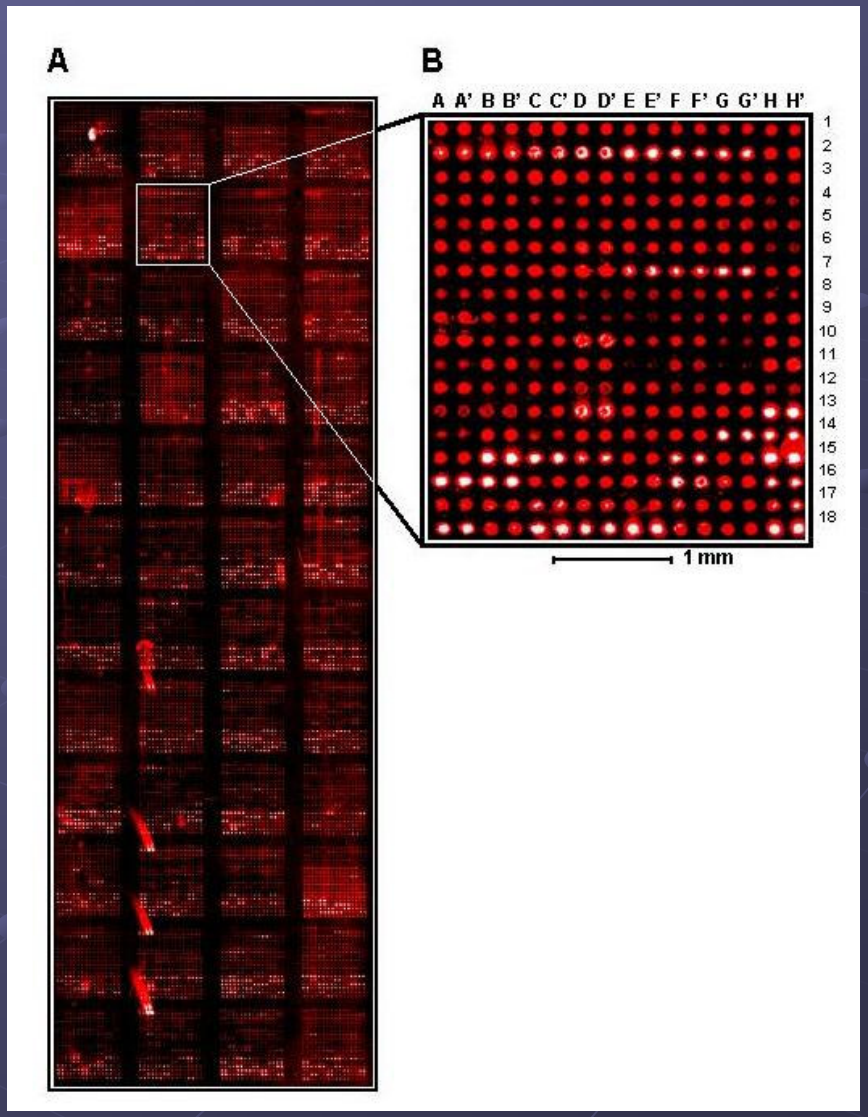
**Indeterminate:** Any bands present but do not meet criteria for positive

# Micro chip array



# Automatization



# References

Introduction to Biotechnology by W.J. Thieman and M.A. Palladino. *Pearson & Benjamin Cummings* 2nd edition.

http://www.toodoc.com/SDS-PAGE-ppt.html

http://www.bio.davidson.edu/courses/genomics/method/Westernblot.html