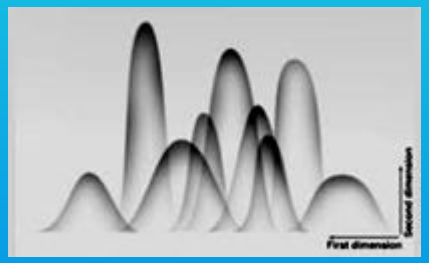
VIDYASAGAR UNIVERSITY

MemoNo. VU/R/Noti/398/2020 Dated 30/03/2020

4. Concept of taxonomic characters:

Serotaxonomy: Its applications in Systematic

Lecture for 4th Semester Special Paper
(BOT 402A) :
Angiosperms Taxonomy and Molecular Systematics



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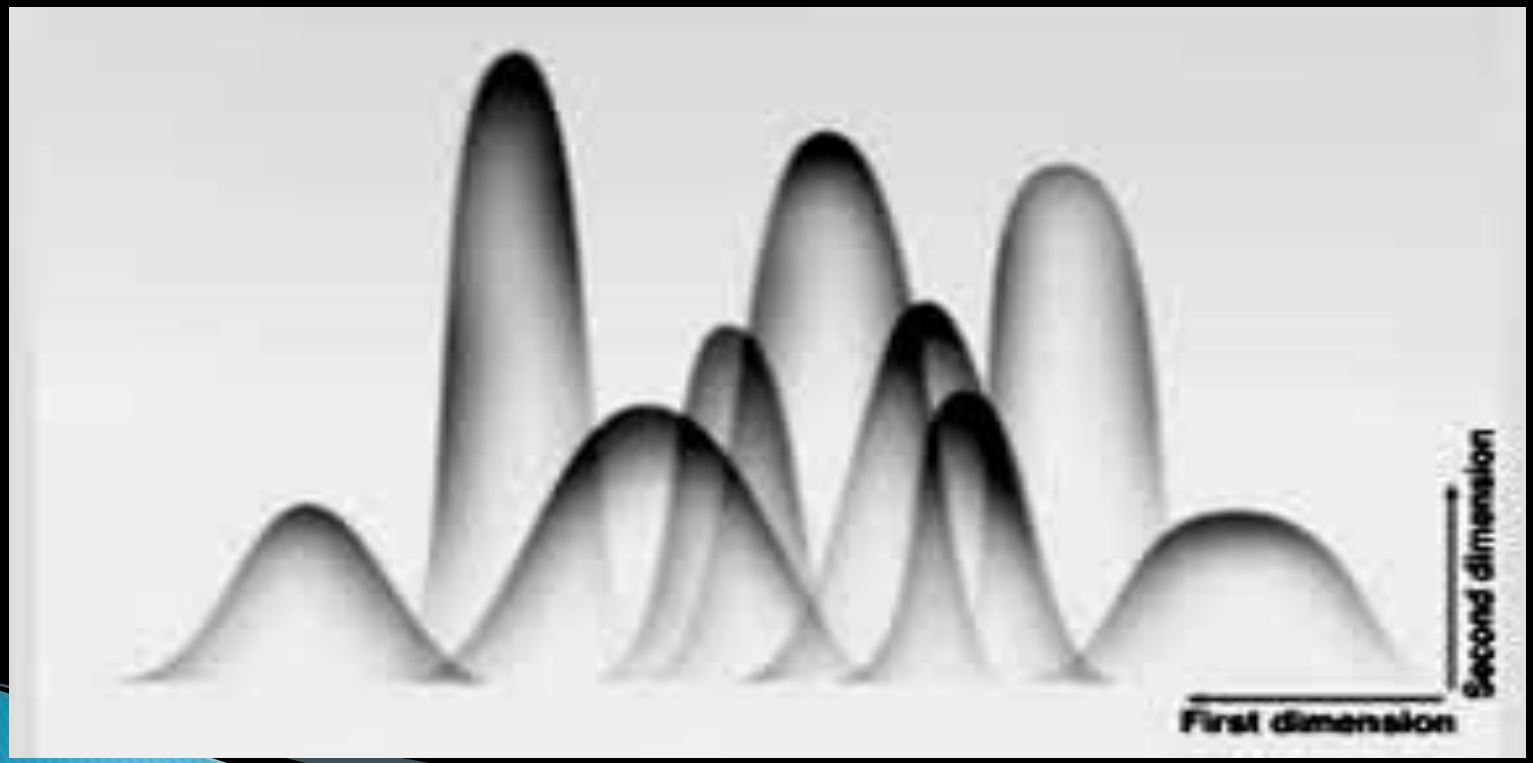
Vidyasagar University

30th March, 2020

Serotaxonomy:

Its applications in Systematic

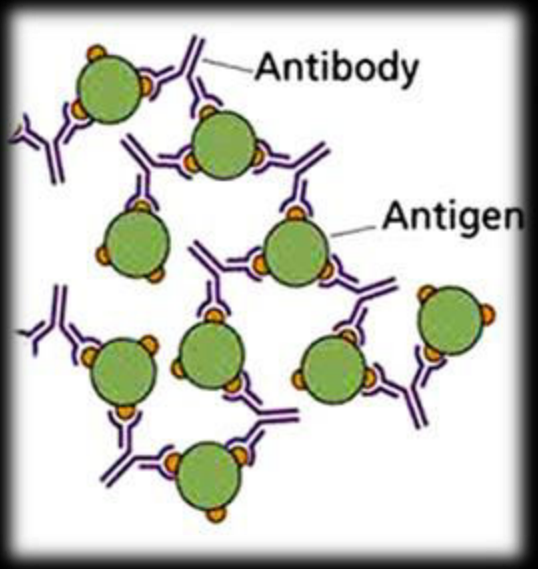


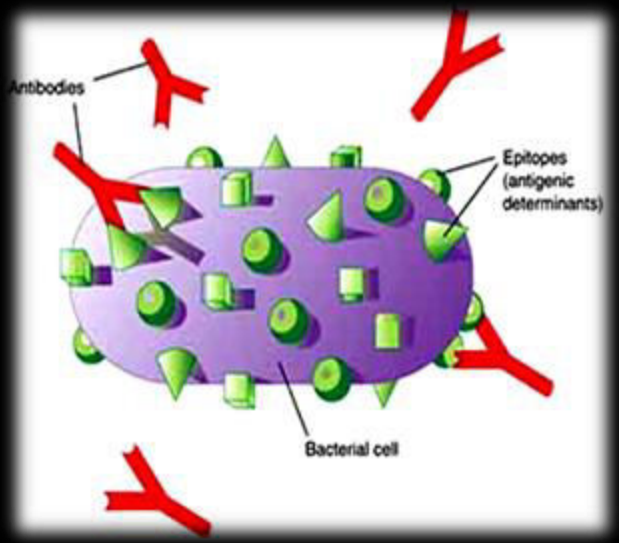
Definition

"The study of the origins and properties of antisera."

When foreign cells or particles (antigens) are introduced into an organism, antibodies are produced in the blood (antiserum).

- The substance capable of stimulating the formation of an antibody is called **antigen**.
- The highly specific protein molecule produced by plasma cells in the immune system in response to the antigen is called **antibody**.





- Proteins most widely used as antigens in serotaxonomy.
- Which carry useful taxonomic information and are easy to handle.
- Both structural and reserve proteins can be used in the field of systematics.
- As long as they belong to the same group and the same organs are always compared.
- Generally, storage proteins are most amenable for taxonomic studies followed by pollen proteins, Stem tubers, algal cells, fern spores, fruits and leaves can also be employed as satisfactory antigenic material for systematic investigations.

Phytoserology

- Phytoserology, which deals with immunochemical reactions, between serum antibodies and antigens, has also established itself as a valid method in systematics, because it helps to detect homologous proteins.
- It uses the specific properties of antisera produced by animals against plant proteins as characters to assess plant relationships.
- Serotaxonomy developed and became popular in Germany, which has been an active center since the beginning of this century.

Production of Antiserum from Plants:

- When a plant extract containing proteins or purified proteins or mixed proteins is injected into a mammal (usually a rabbit), it initiates the formation of **antibodies**.

- These are proteinous and specific to that antigen, and are thus capable of rendering it non-functional.

- The antibodies formed are extracted (antiserum) and standardized by testing for sensitivity against a serial dilution of antigen This 'standard' antiserum is used in sero-taxonomical experiments.
- Water-soluble seed proteins are extensively used for this purpose.

Initiation of an Immunological Reaction in Plants:

- **Antiserum gives a precipitation reaction with the plant extract (antigen-antibody reaction).**

- **Similarity of other species to the first one can be assessed by measuring the amount of coagulation it causes. For example, the extracts of related plants will react similarly, although probably to a lesser extent.**
- **But, the extracts from unrelated plants will fail to give a reaction, as they do not contain the same or similar protein.**

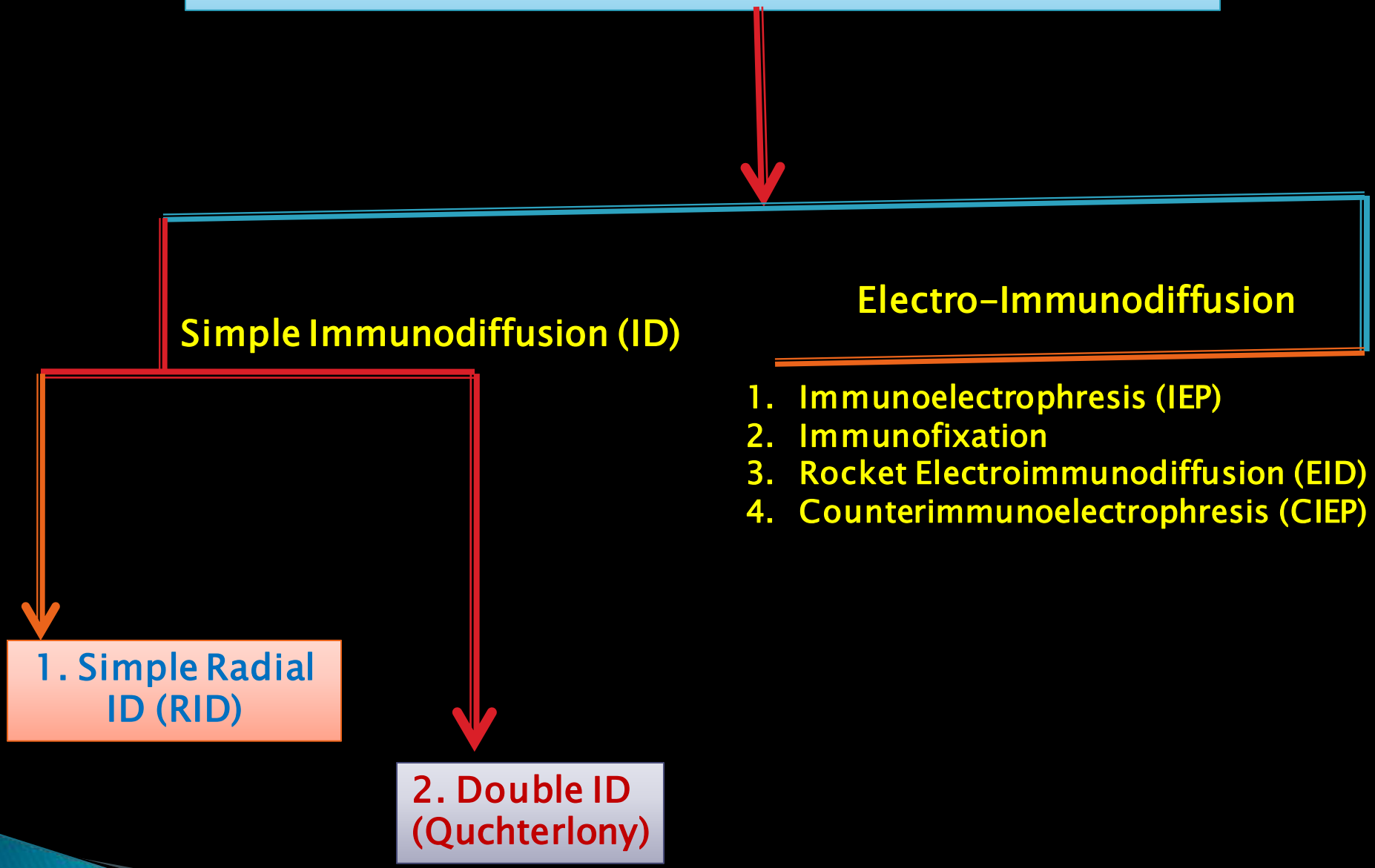
Methods Used in Serotaxonomy:

(a) Immuno-diffusion in Agarose Gels or Gel Diffusion Method:

- In this method the antigen-antibody reaction is carried out in gels, mostly of agarose, in petridishes.
- The antiserum containing antibodies is filled in a well at the centre of the gel and the antigens from related taxa are placed in outer or radial wells.

The antigen and antibody react to produce the insoluble antigen-antibody complex, forming a thin immobile band of precipitin (protein) at equilibrium, which can be visualized either directly or after protein staining for interpretation.

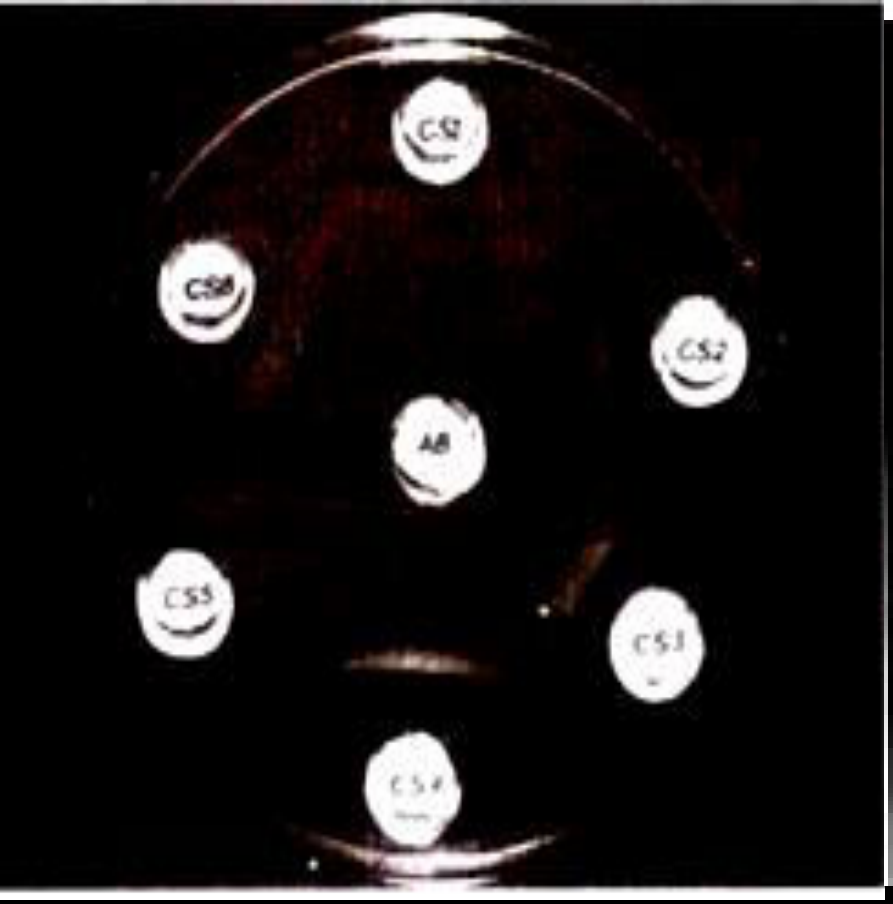
Precipitation reactions (Immunology)



Immuno-diffusion in gels can be further of two types:

i. Single radial immuno-diffusion: In this technique, the antigen is usually allowed to diffuse into the gel containing the antiserum.

ii. Ouchterlony – double immuno-diffusion:



Ouchterlony-Double immunodiffusion between the various antigenic proteins of the pollen of *Cassia siamea* (CS-1-CS-11) in the (outer walls) and the antibody (AB in the inner wall) against the total protein from the same pollen.

In this method, both the antigen and antibody are allowed to diffuse into the gel and meet each other.

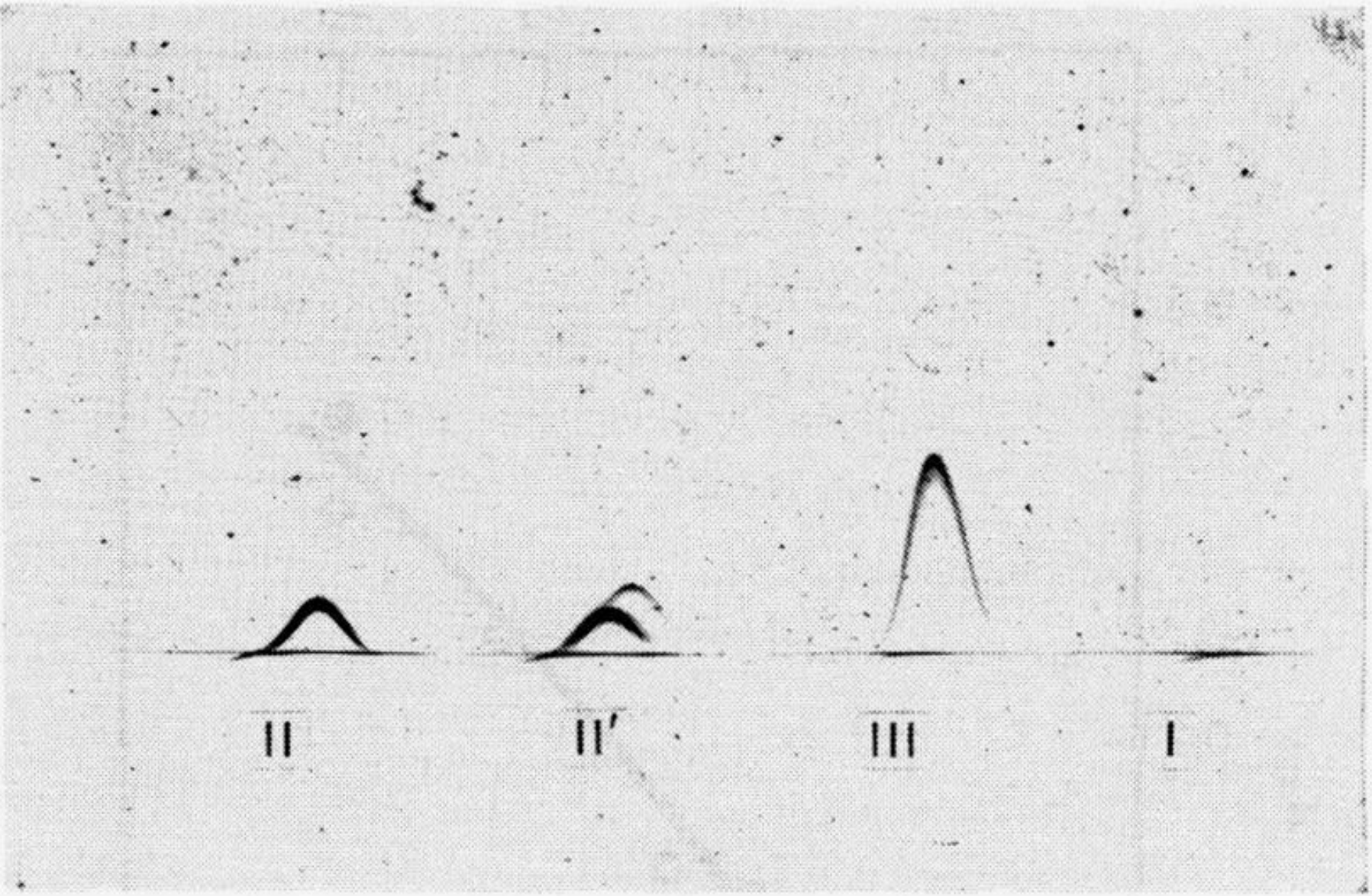
(b) Rocket Immunoelectrophoresis:

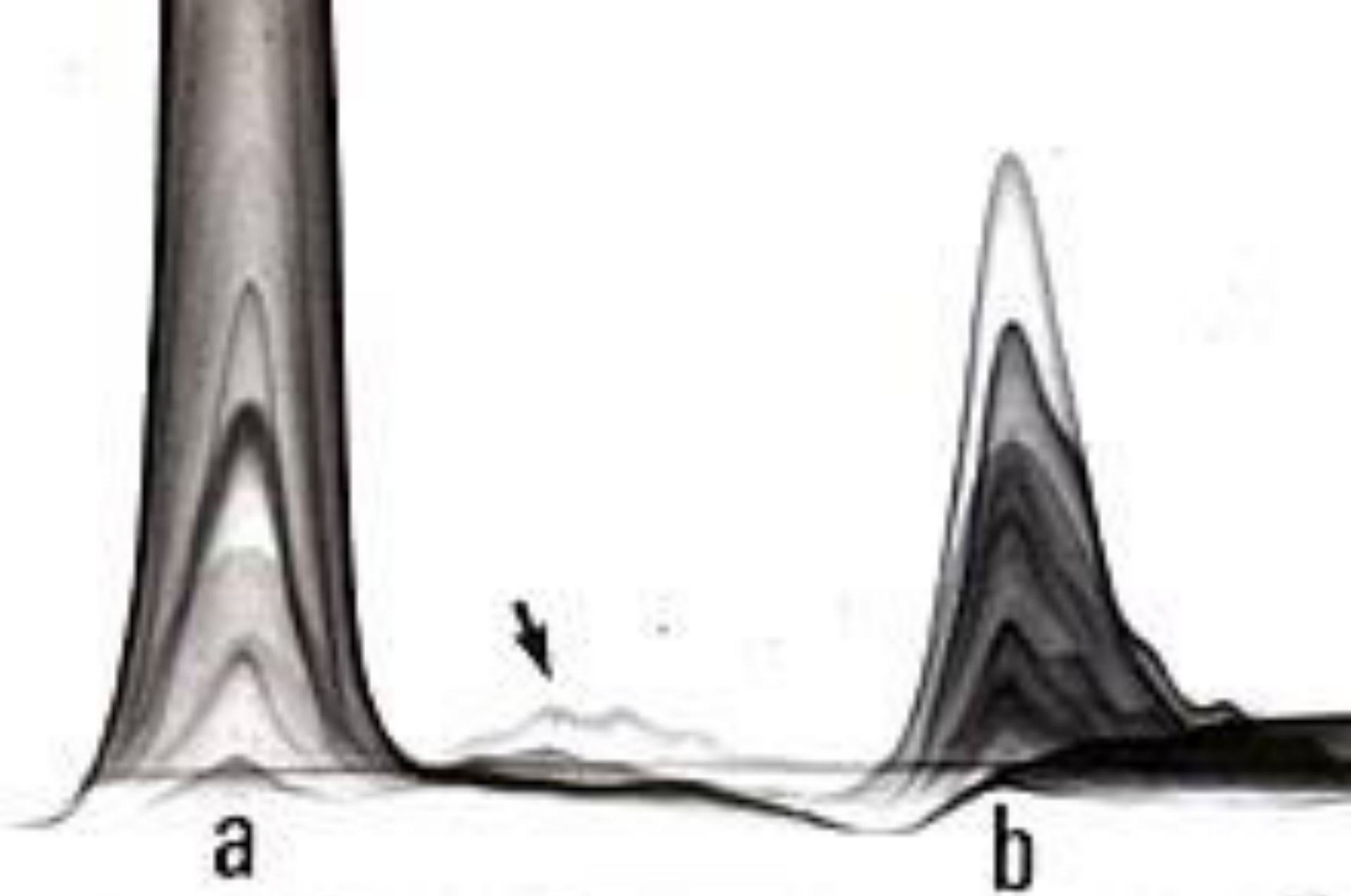
- It is a simple, rapid and reliable method in which, rocket-like immunoprecipitate is formed when the desired protein (antigen) is electrophoresed in an agarose gel containing its mono-specific antiserum.

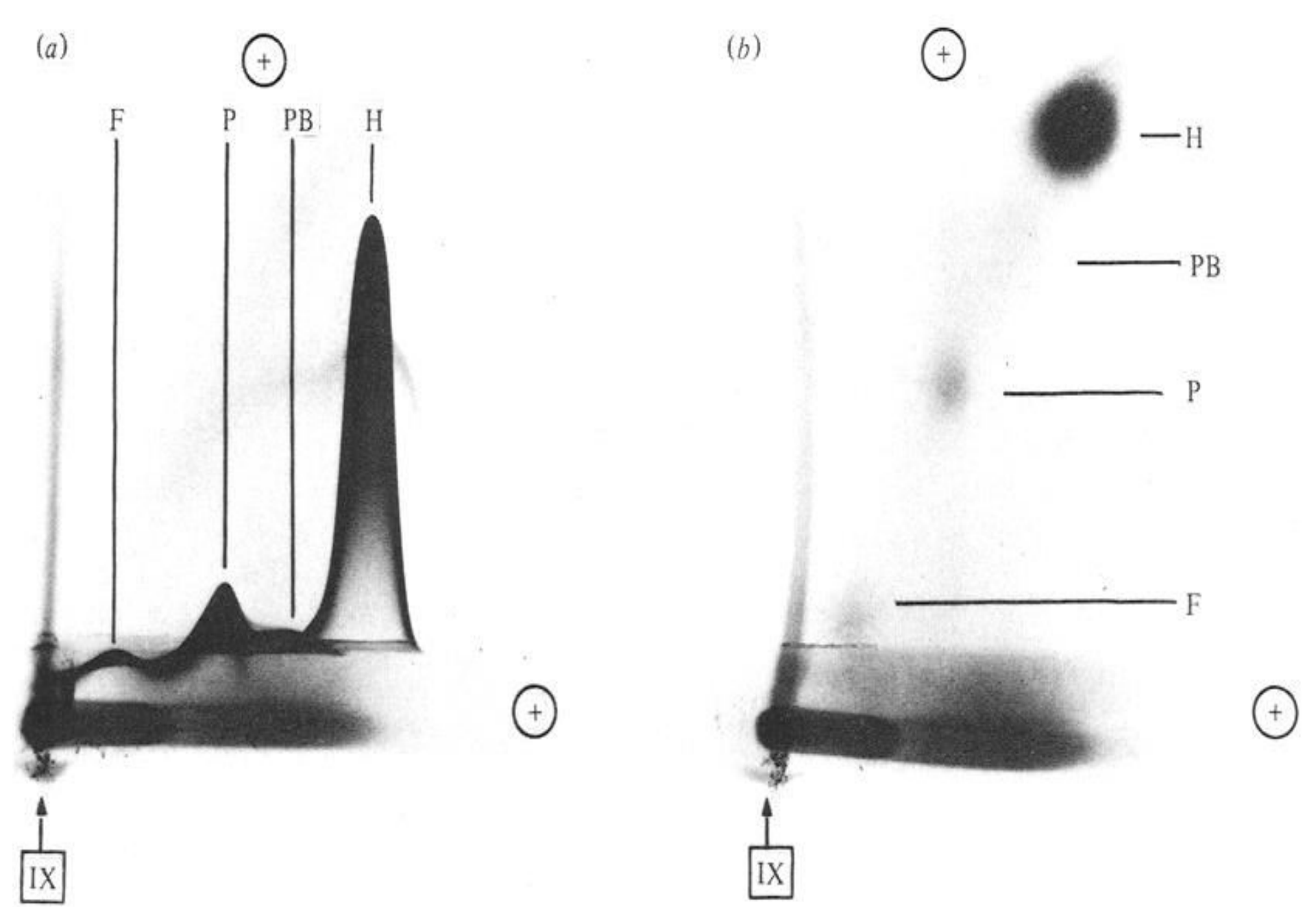
- A comparison of the height of the peaks of the unknown and standard samples also allows the unknown protein concentration to be determined.

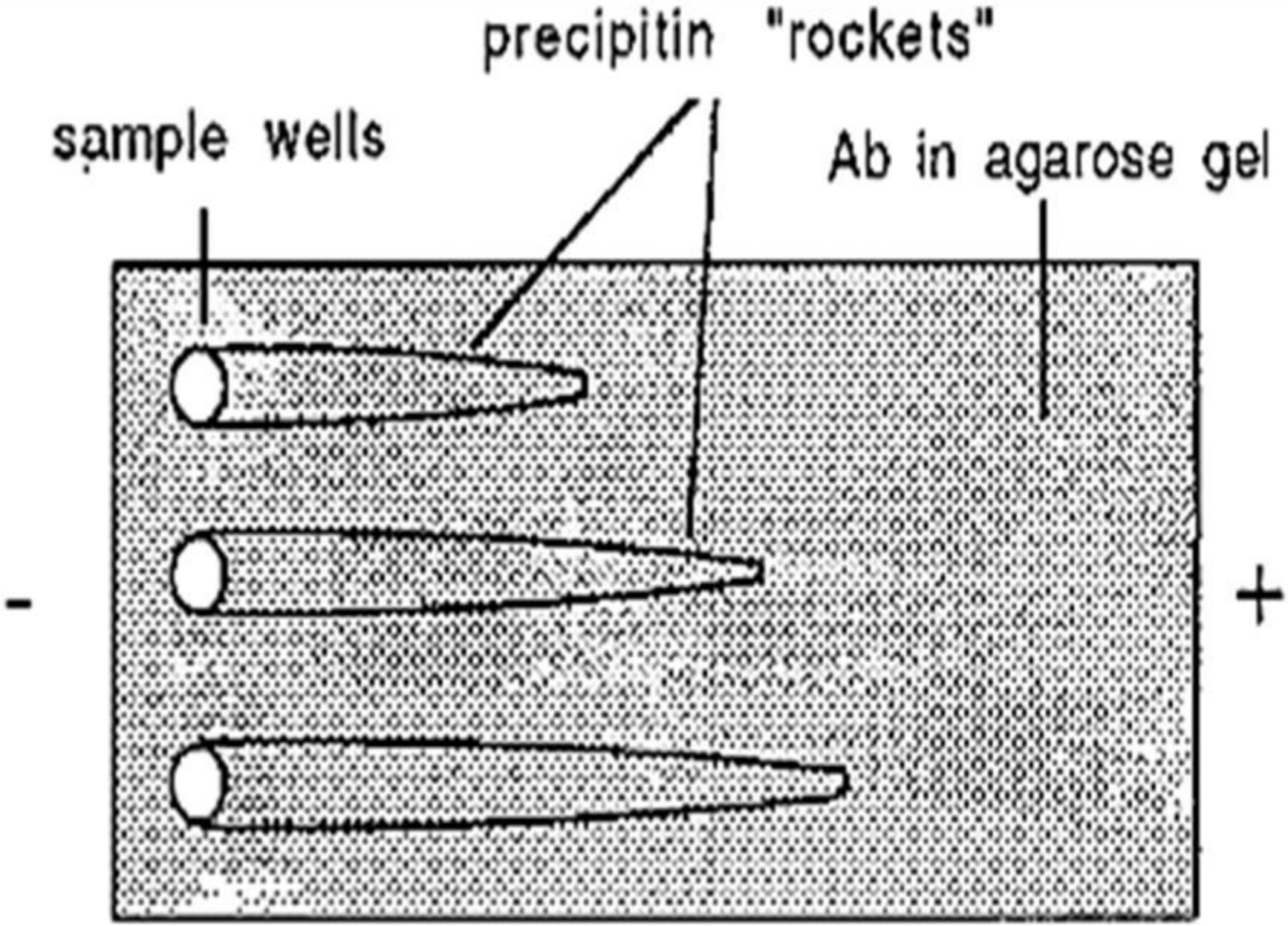
(b) Rocket Electrophoresis:

- Crossed Immunoelectrophoresis of antigens and antiserum.
- In the first dimention, proteins are separated by standard electrophoresis.
- The separated proteins are then run into the second dimension gel at an angle of 90 degree from the first dimension.


II
II′
III
I
Second dimension (immunoelectrophoresis) →
–
First dimension (electrophoresis) →
+

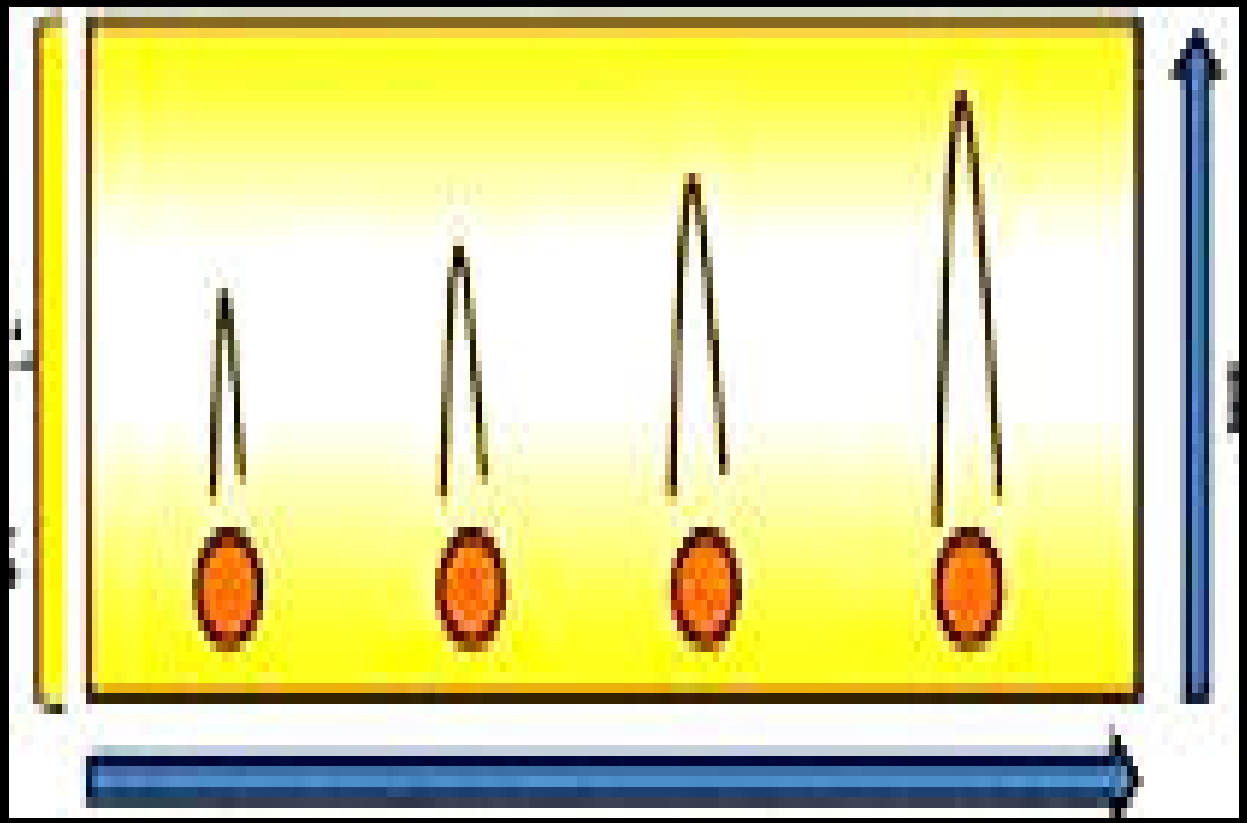

a
b


(a)
+
F
P
PB
H
+
IX
(b)
+
H
PB
P
F
+
IX


precipitin "rockets"
sample wells
Ab in agarose gel
-
+

ANTIBODY INCORPORATED IN THE AGER

Agar Gel Poured on Glass Slide

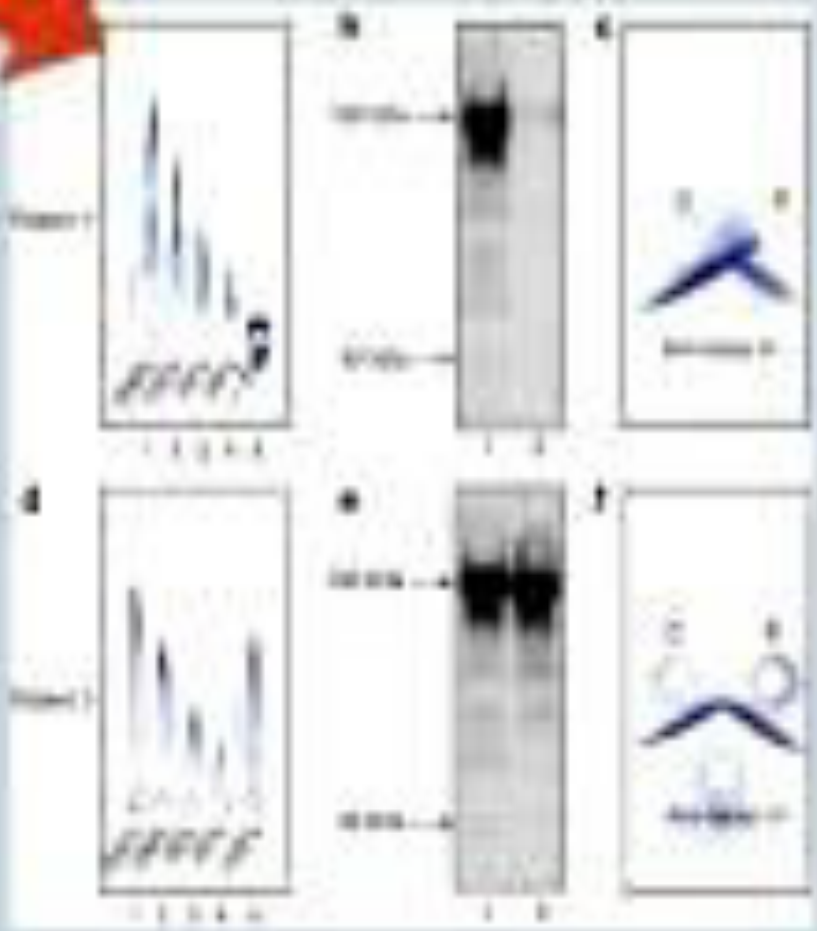


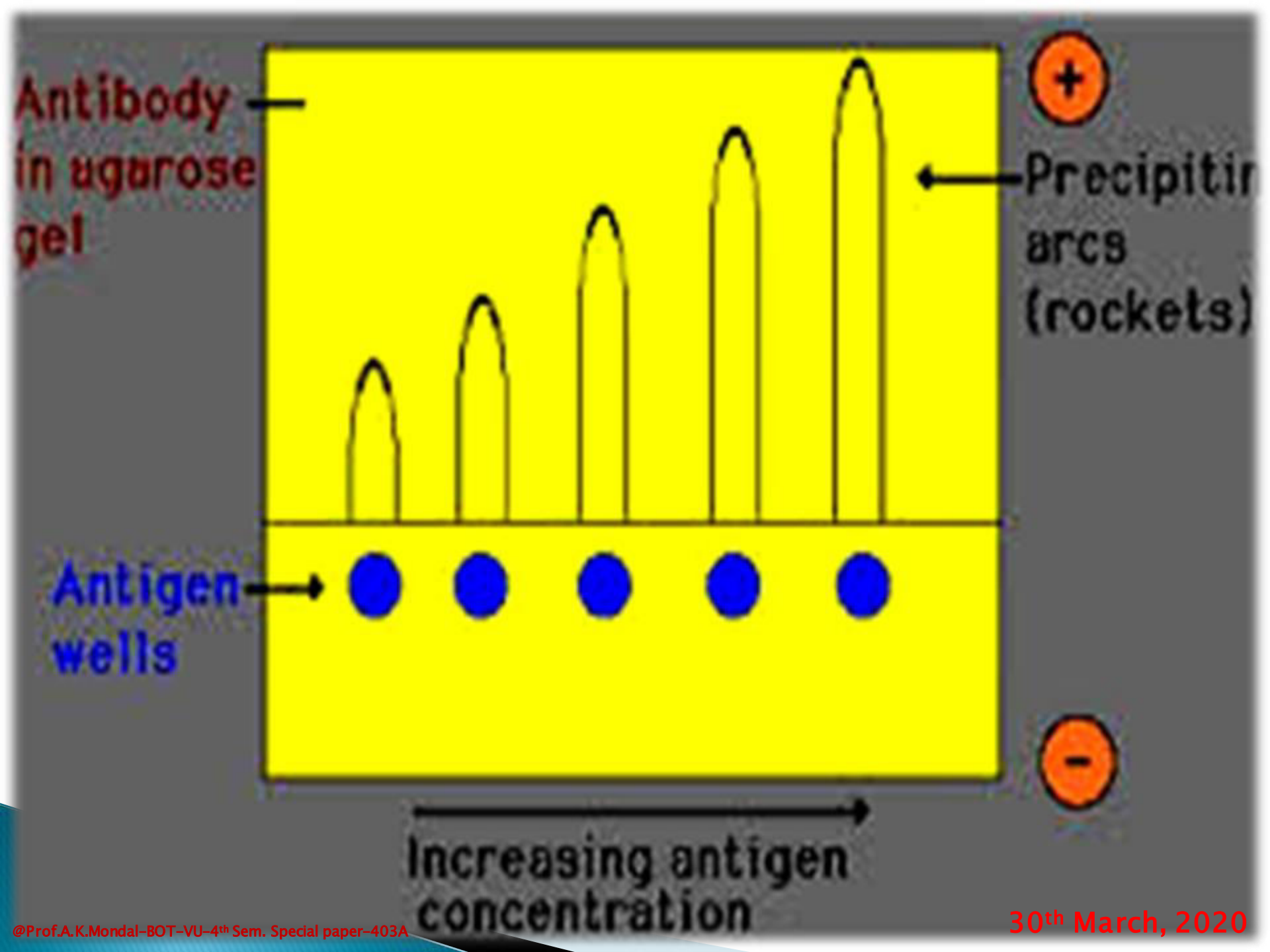
Height of precipitation line

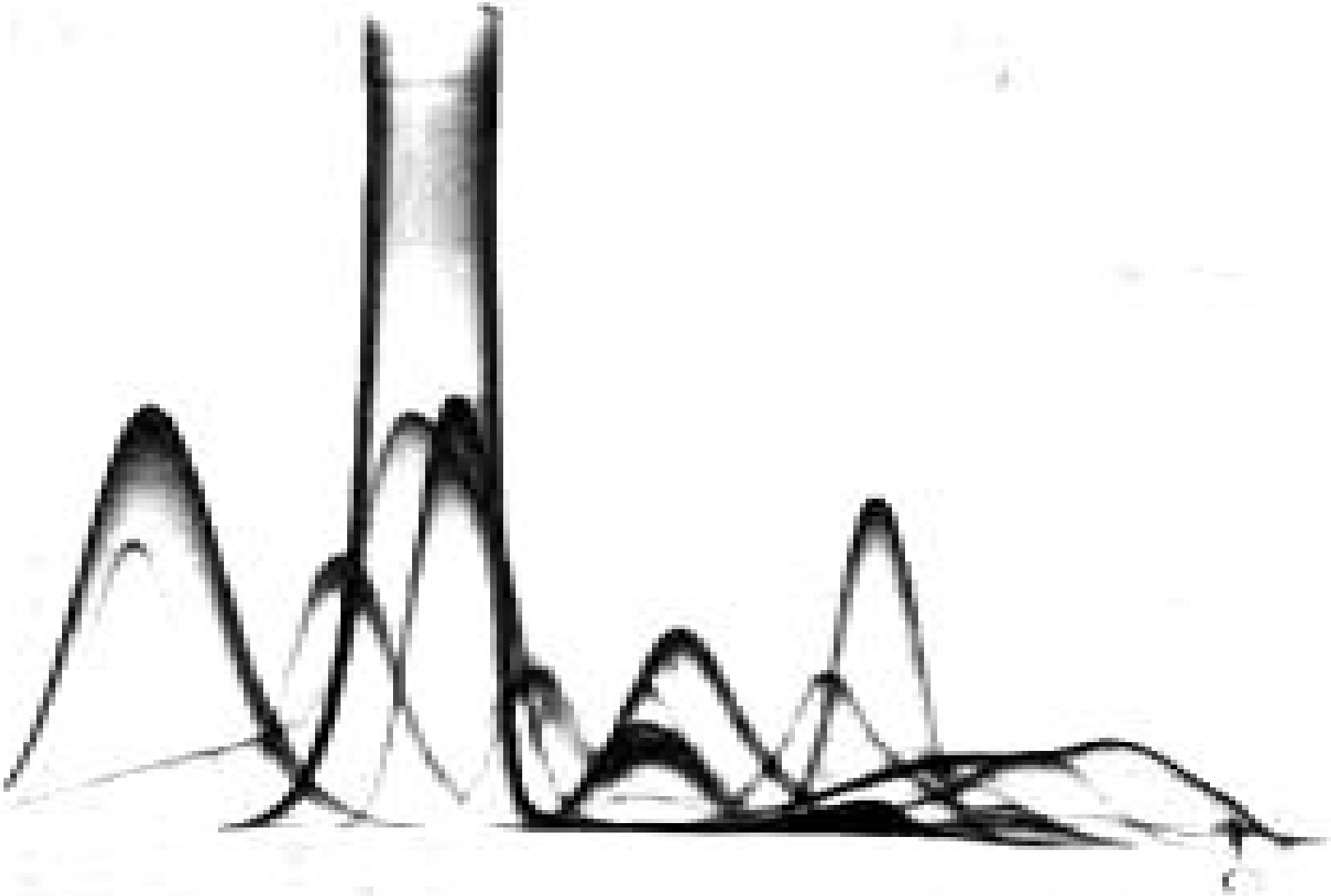
Increasing Antigen Concentration

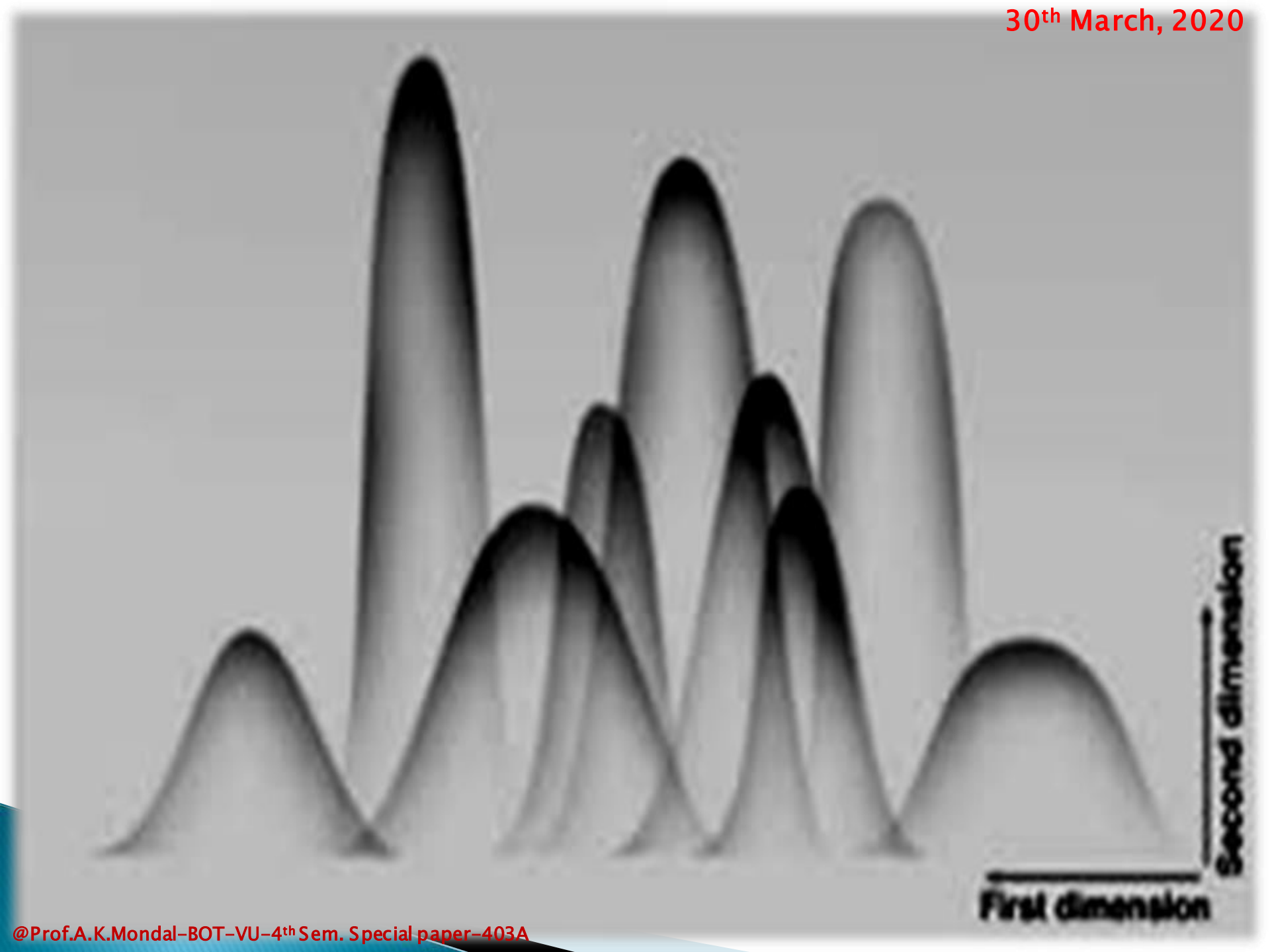
Rocket Electrophoresis

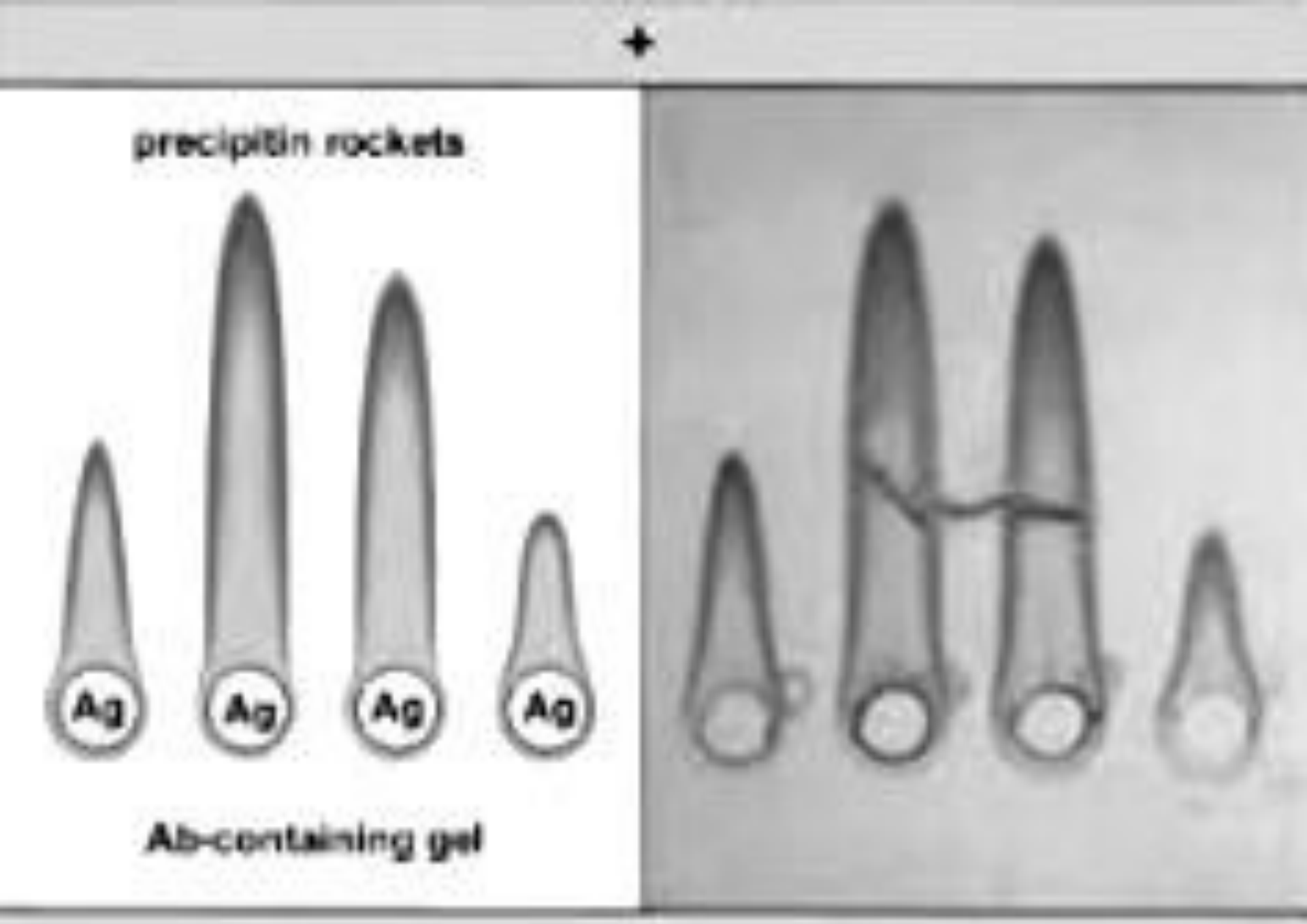
- Adaptation of radial immunodiffusion (RID).
- Antibody incorporated (mixed) into the gel.
- Antigen added to wells.
- **Apply electrical current** and antigen will move forward and will bind to antigen.
- Dissolution and reformation occurs.
- Height of precipitin band related to concentration of antigen.
- Much faster than RID

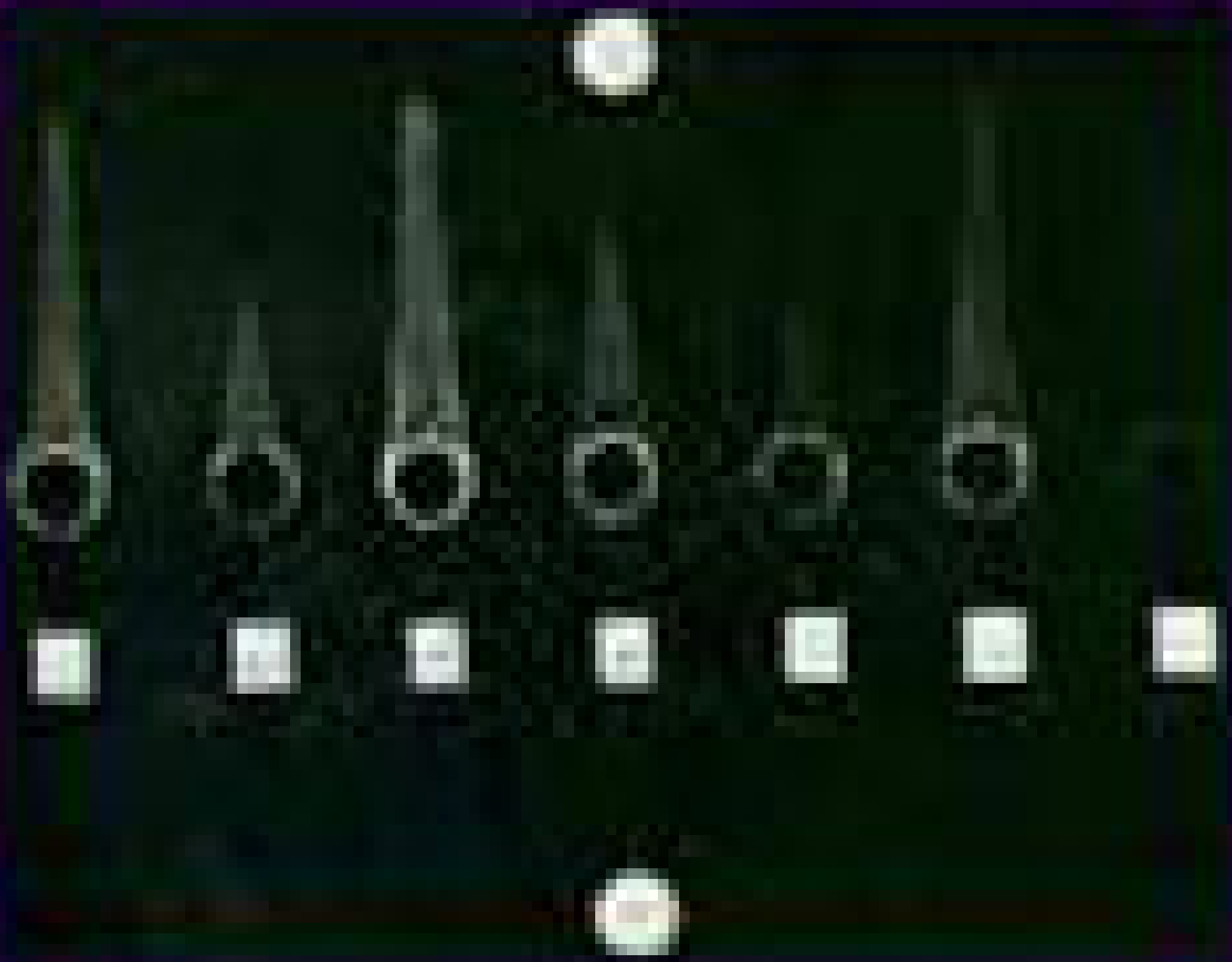


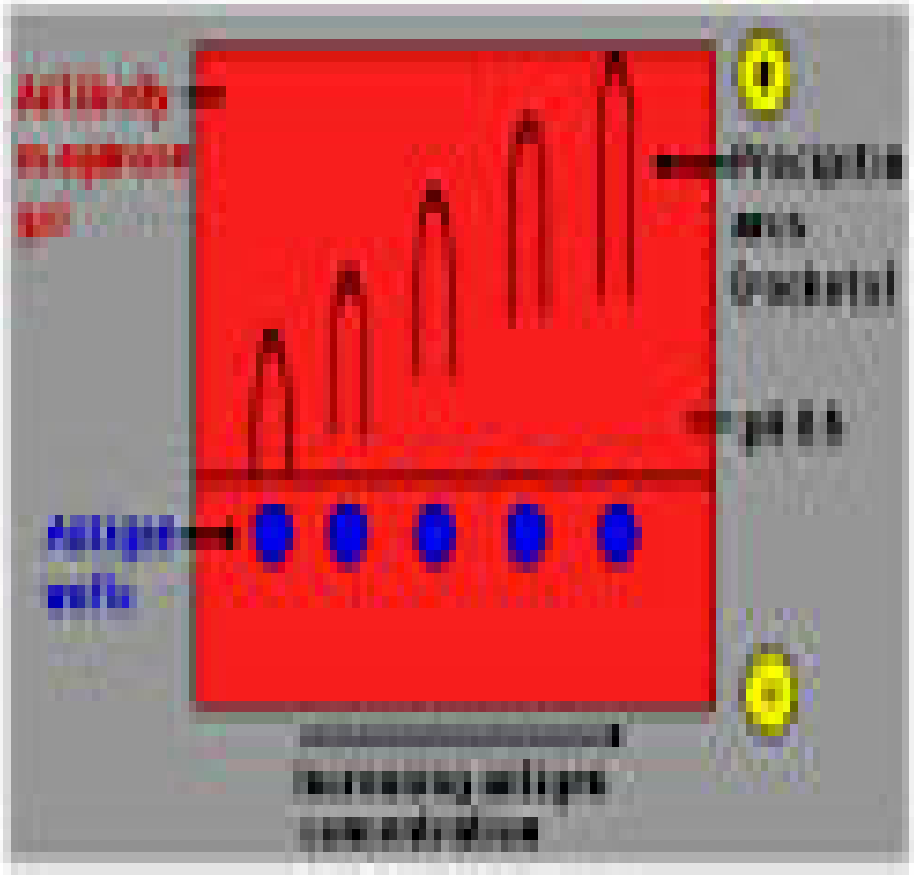

Antibody in agarose gel
Precipitin arcs (rockets)
+
Antigen wells
-
Increasing antigen concentration

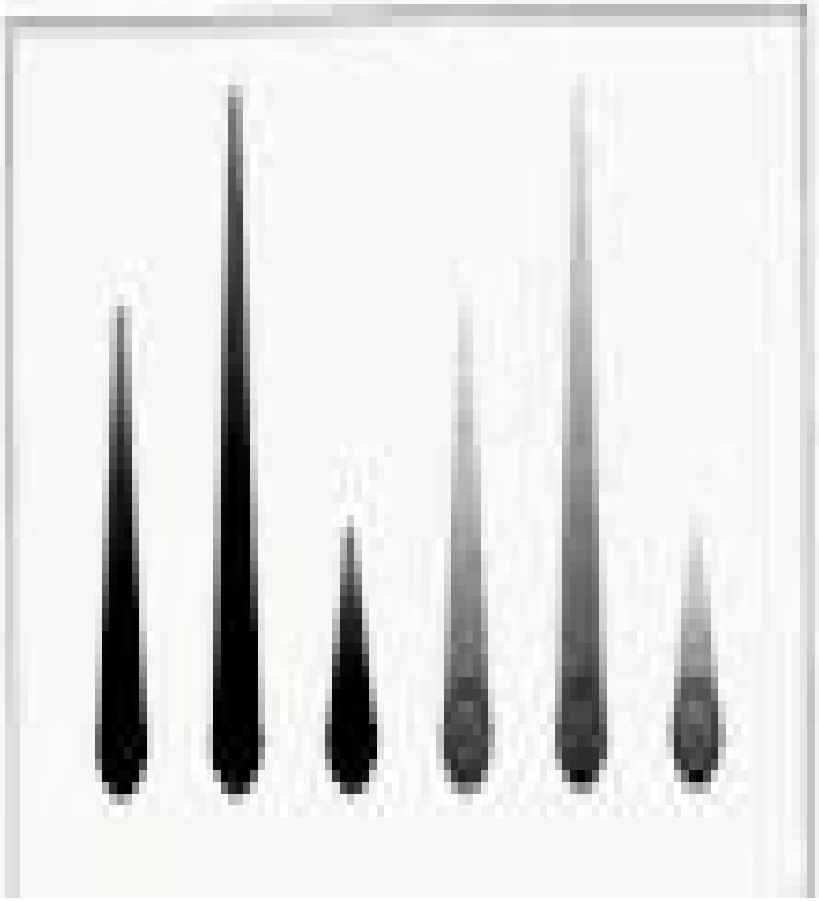



Second dimension
First dimension


+
precipitin rockets
Ag
Ag
Ag
Ag
Ab-containing gel
-

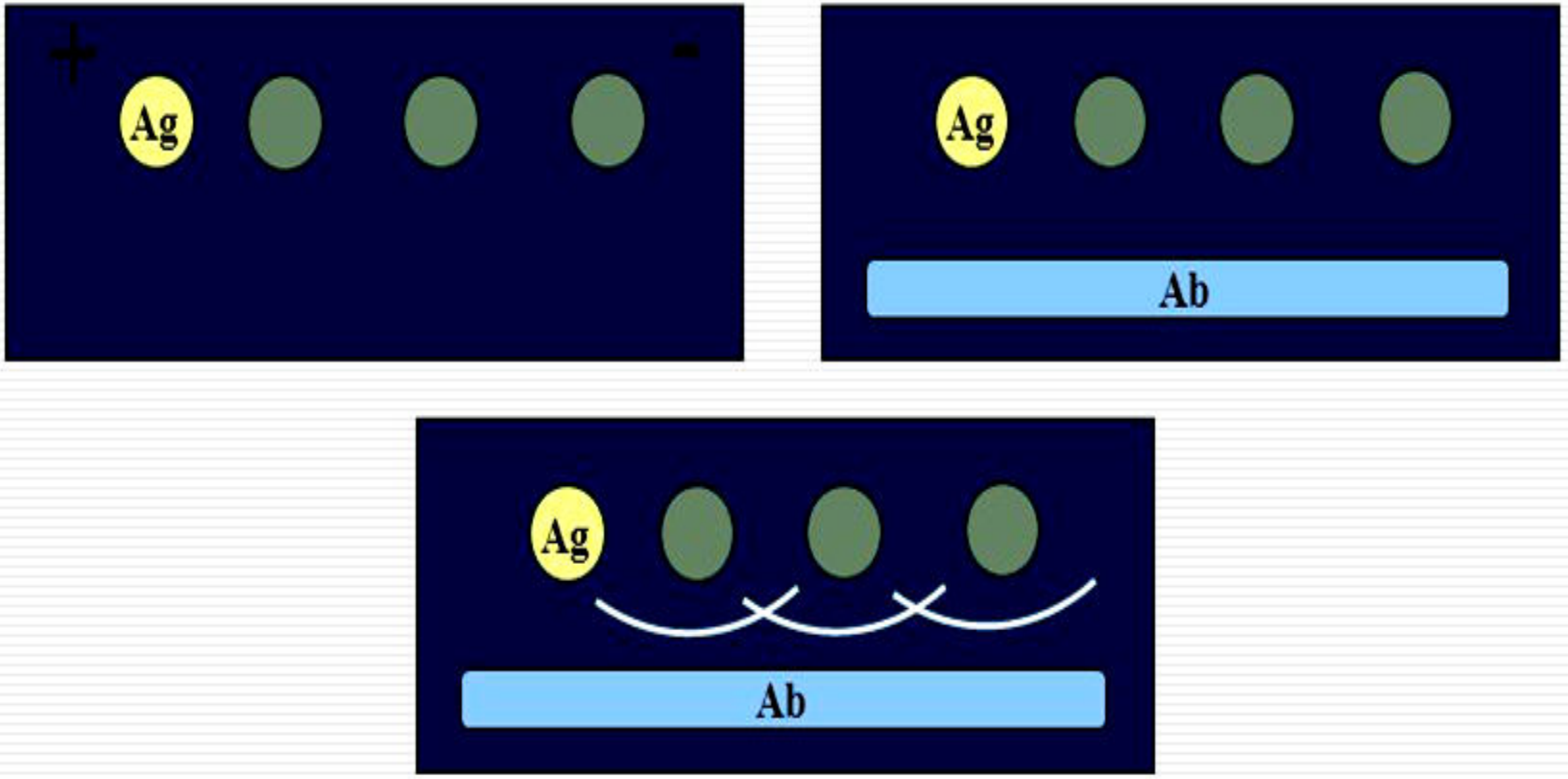






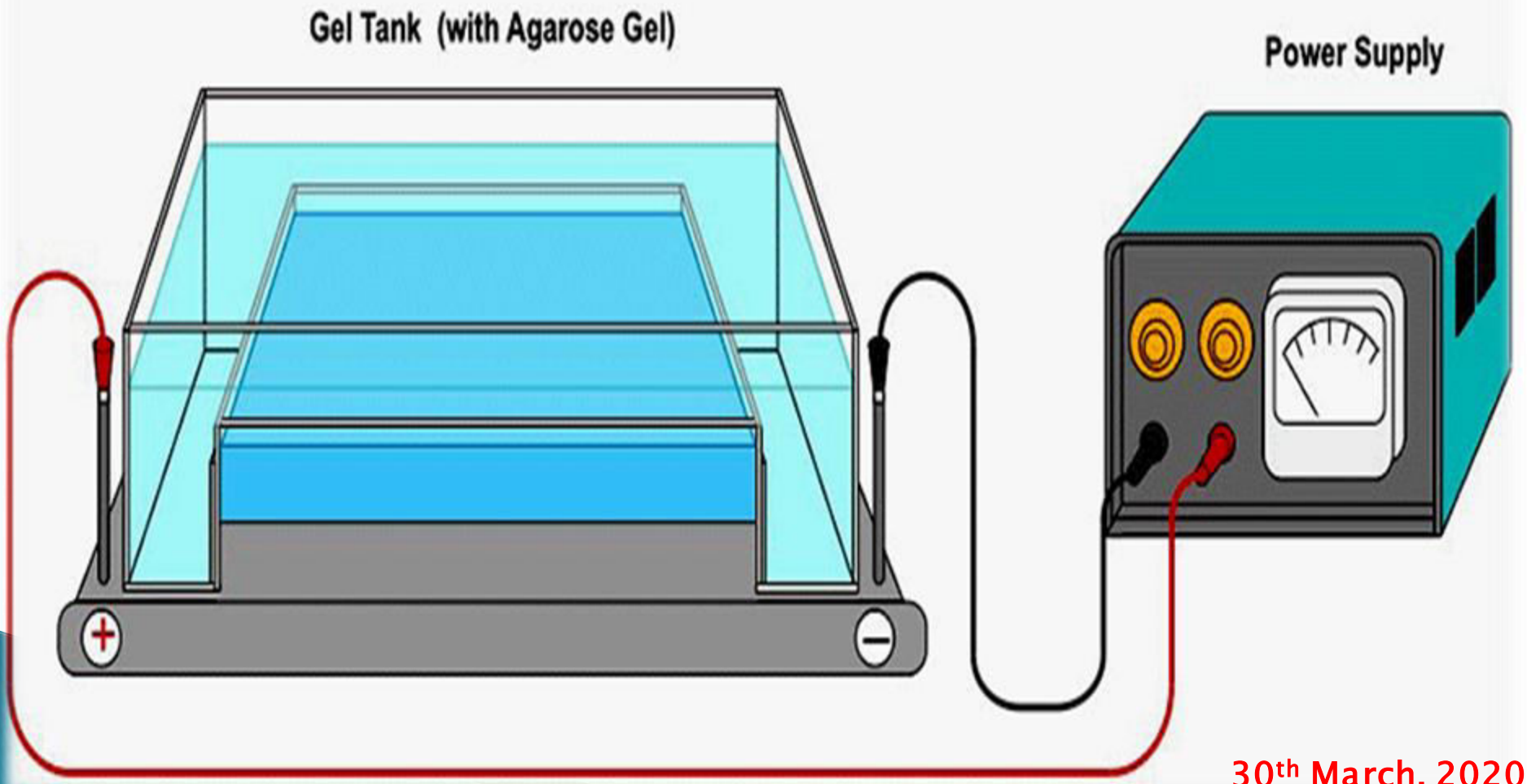
Antigen is electrophoreses into gel containing antibody. The distance from the starting well to the front of the rocket shaped are in related to antigen concentration.

- Method
  - Ags are separated by electrophoresis



- Interpretation
  - Precipitin arc represent individual antigens

Immunoelectrophoresis



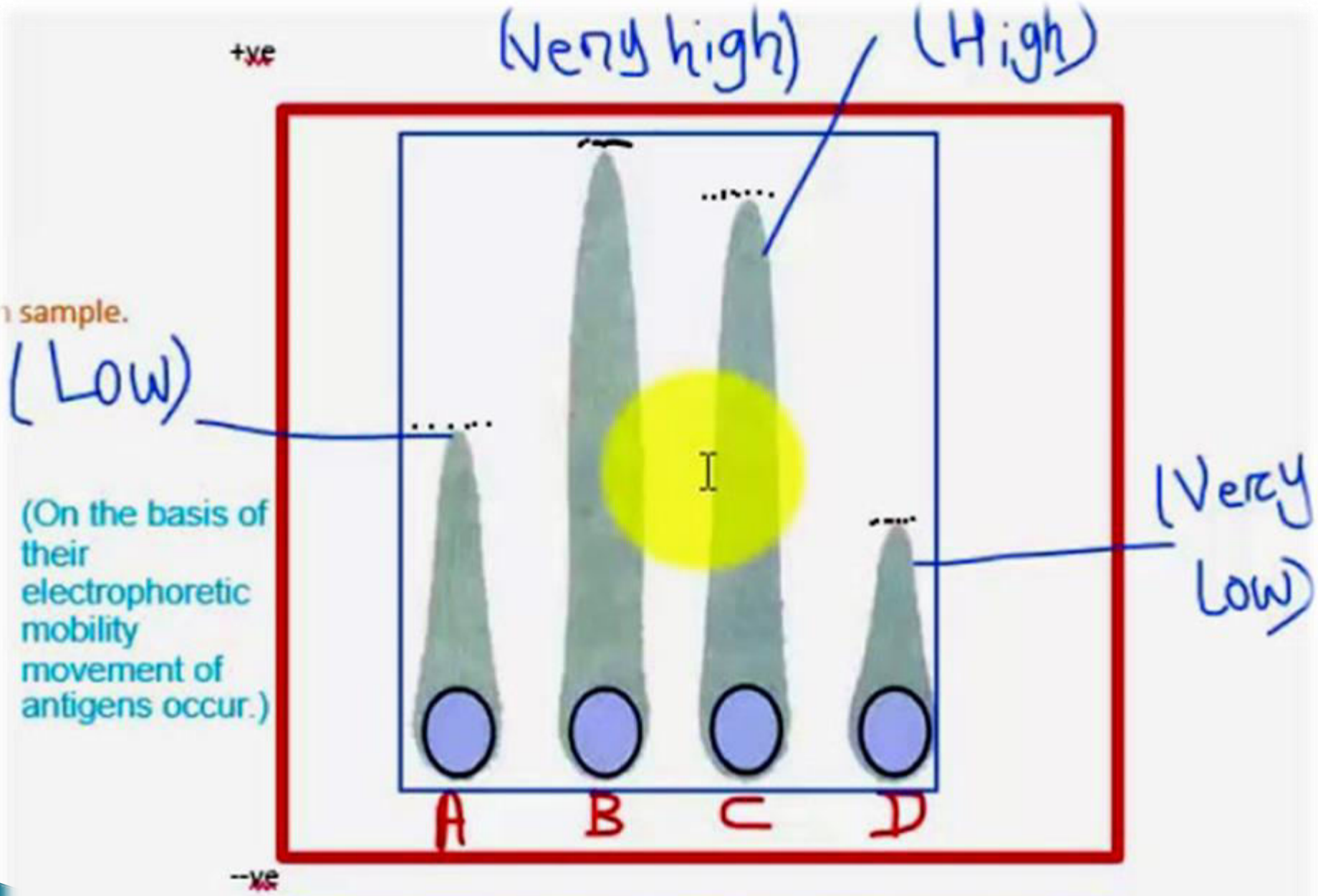
PRINCIPLE- Method of determining the concentration of antigen (Ag) in an unknow

PROCEDURE-

1. Make the agarose(purified agar) solution.
2. Add antiserum .
3. Pour the mixture into the glass plate .
4. Allow to solidify .
5. Place the glass plate in electrophoresis tank after puring the buffer.
6. Make some wells by puncturing with gel puncher without damaging the gel.
7. Load the antigens in the well.
8. Electrophorese the samples till the rockets are visible at 100 v.
9. Measure the height of the rocket and note the observation .
10. From the peak height concentration of antigen is determined.

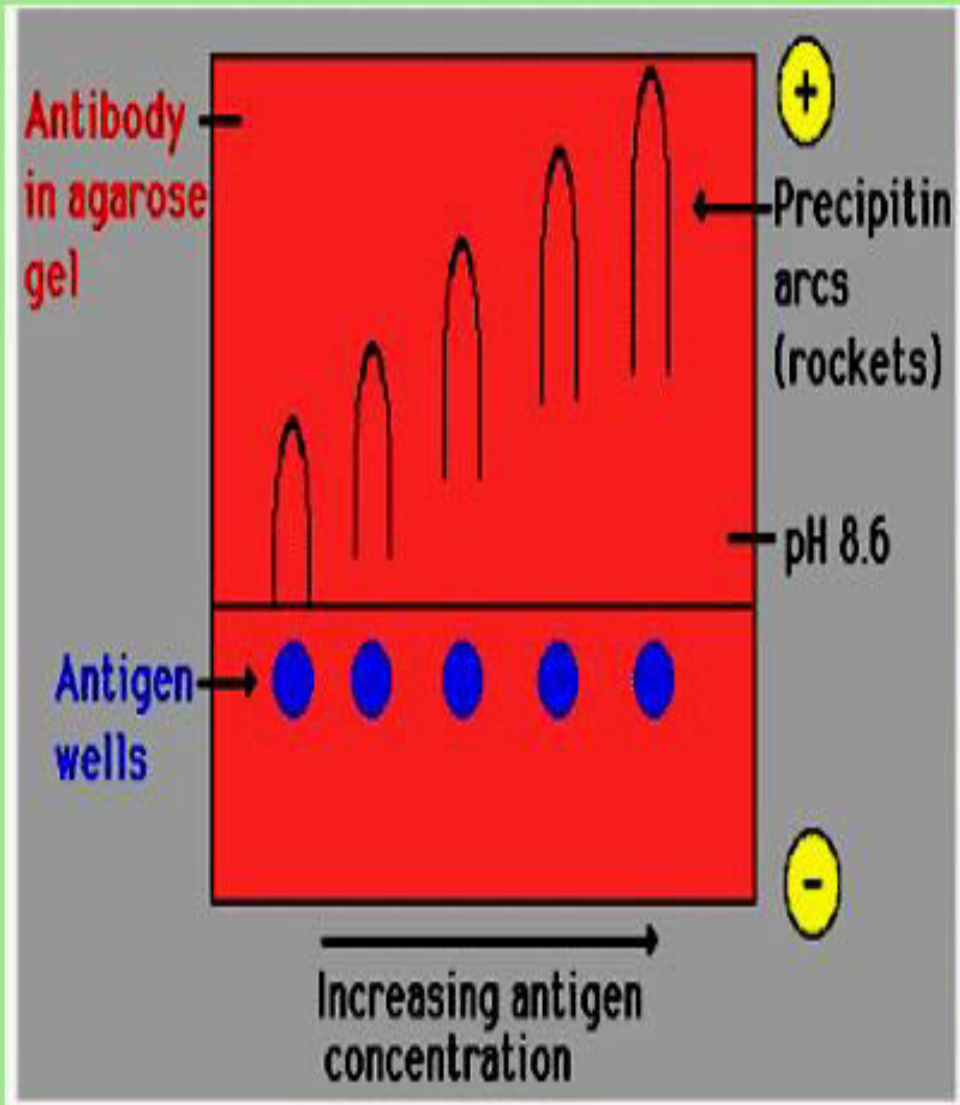
Conclusion -

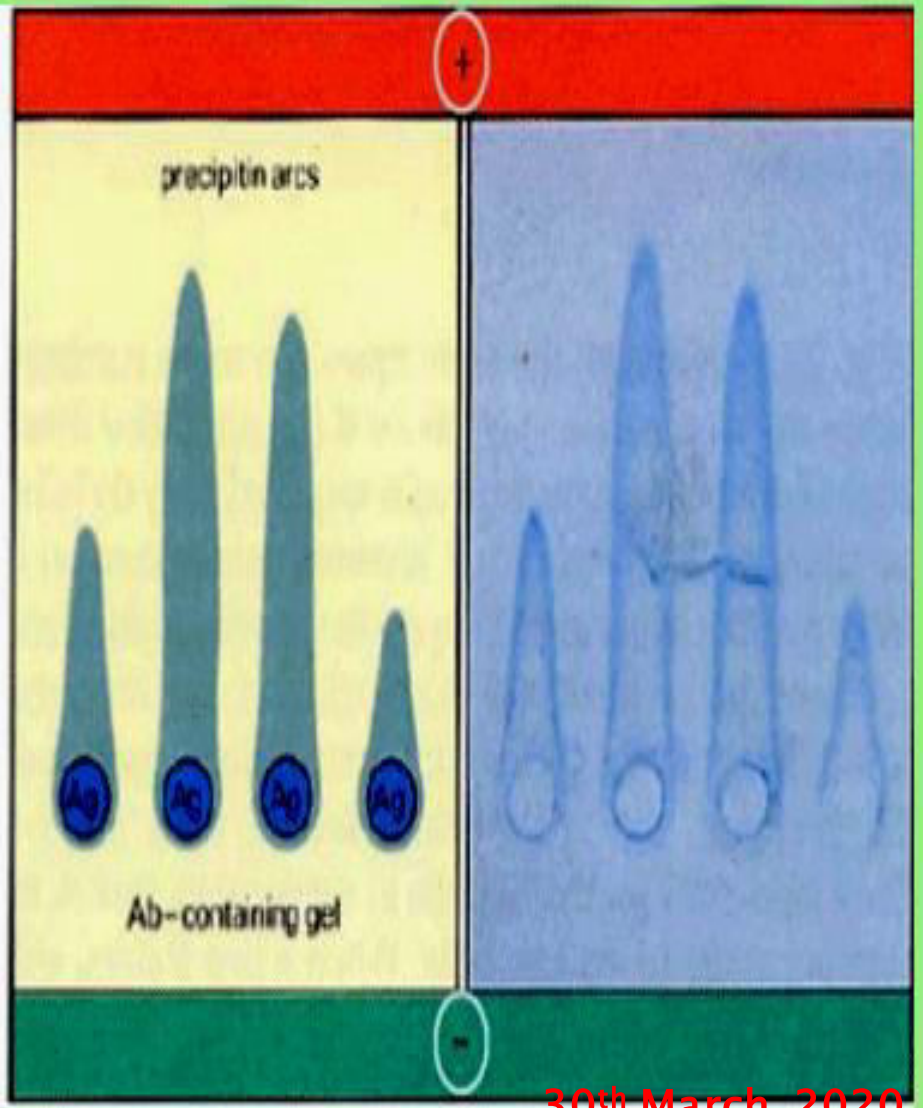
The majority of the antibody-antigen precipitate is indeed at the head of the rocket



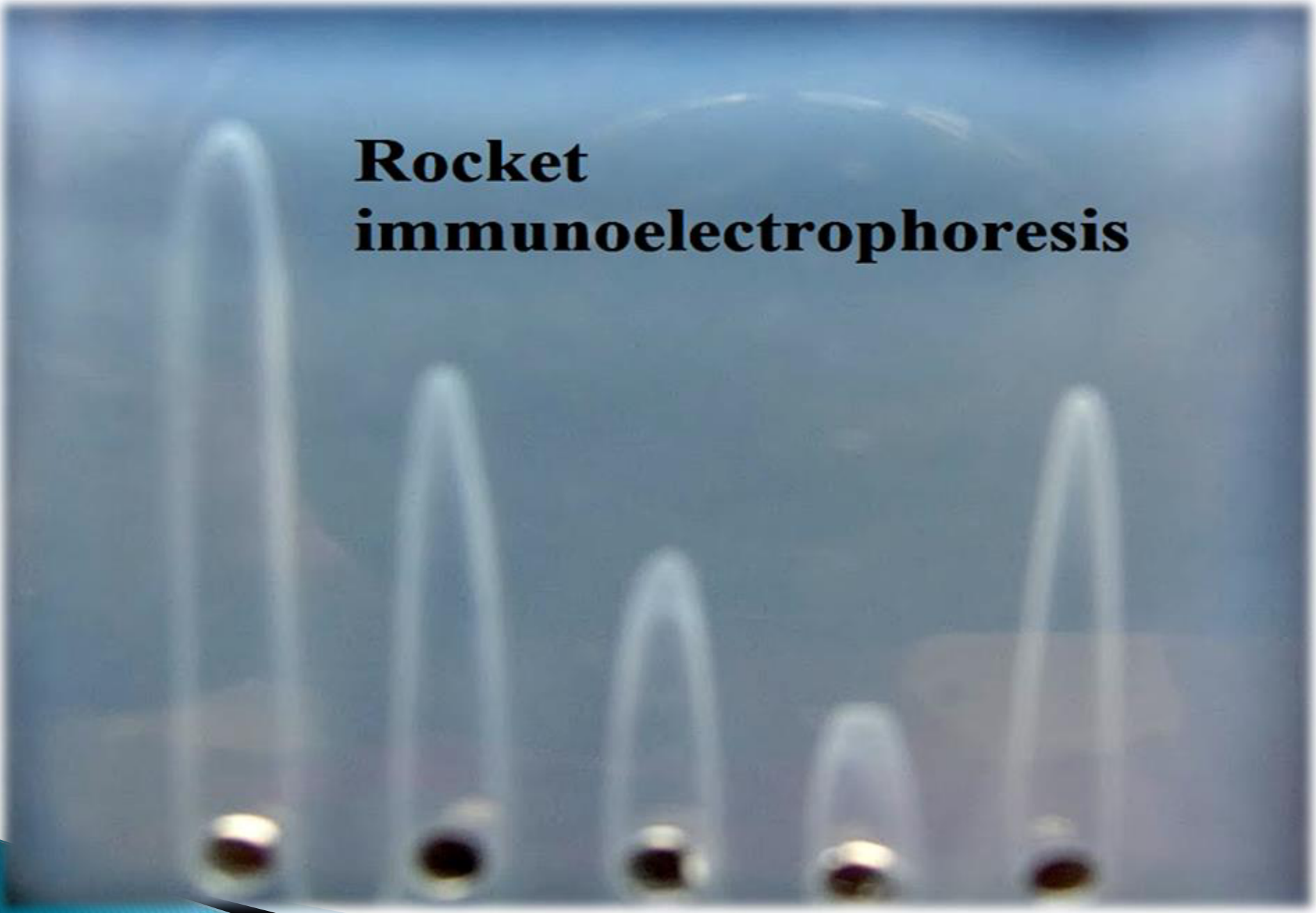
Antigen is electrophoresed into gel containing antibody. The distance from the starting well to the front of the rocket shaped arc is related to antigen concentration.

Rocket Immunoelectrophoresis





Rocket immunoelectrophoresis

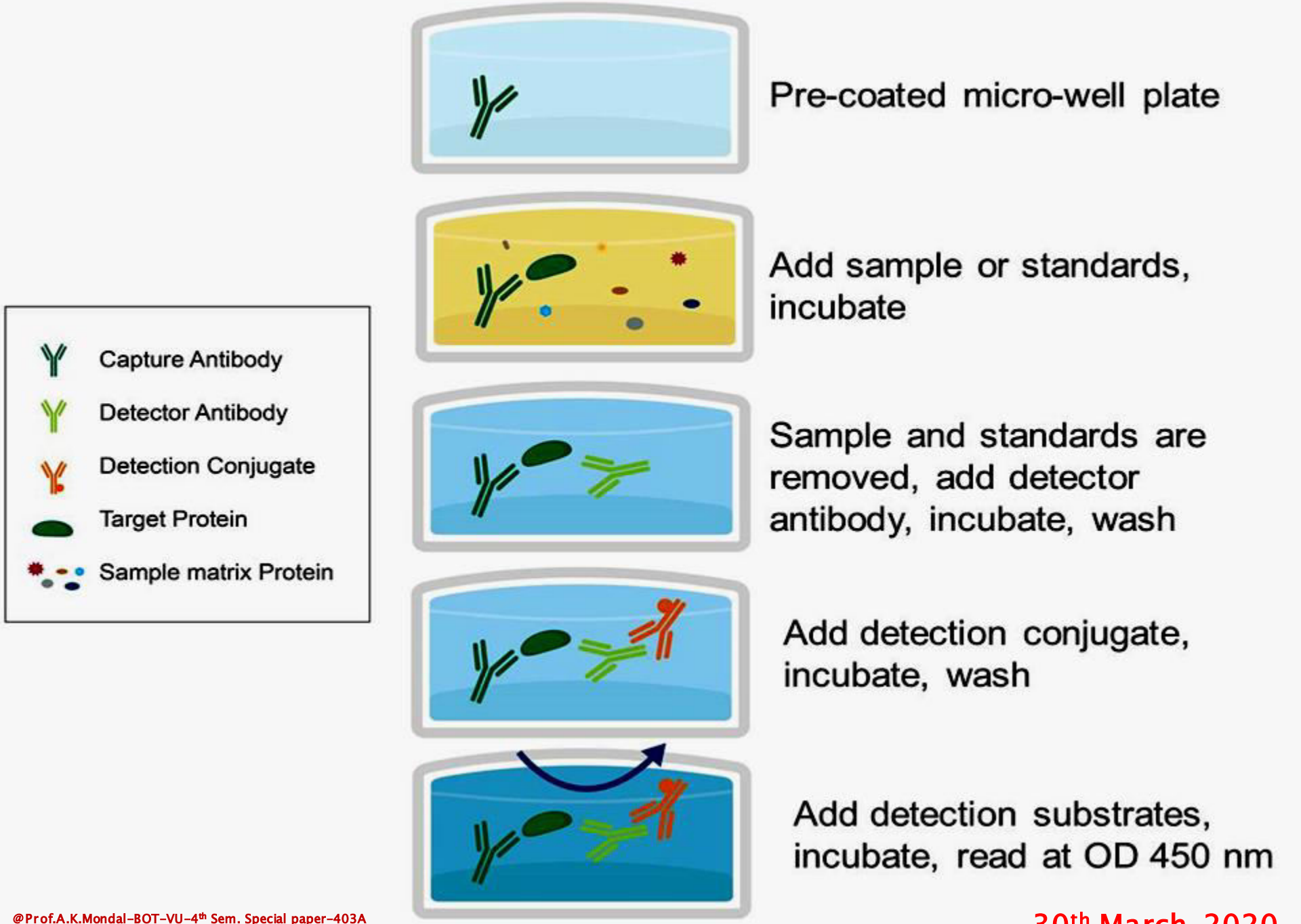


(c) Enzyme-Linked Immunosorbant Assay (ELISA) :

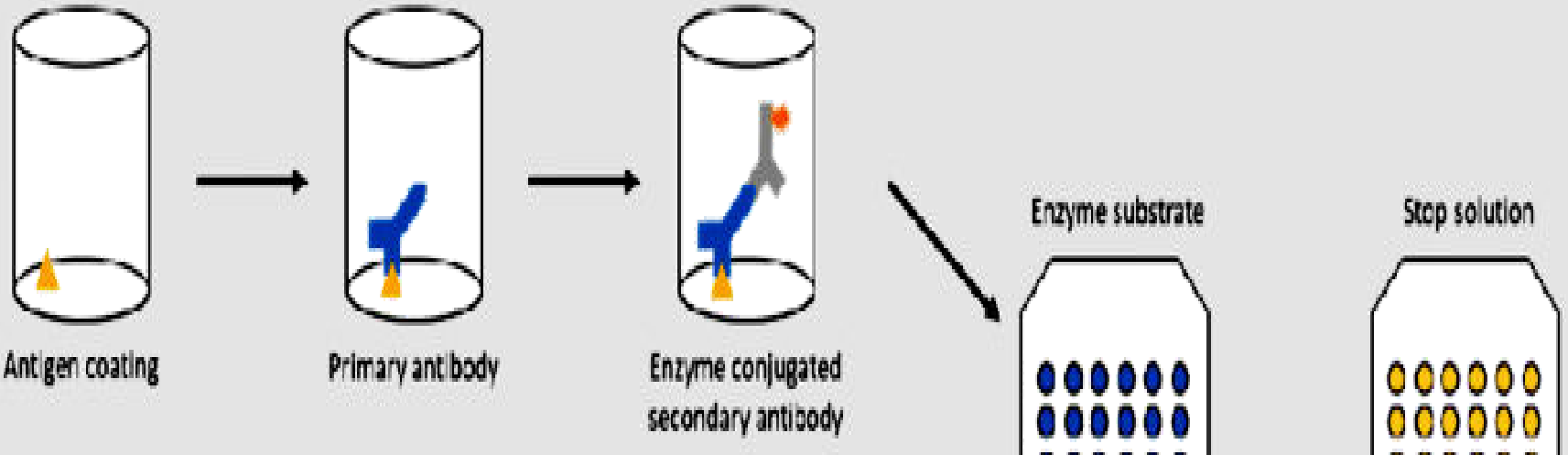
This method is generally used for quantitative estimation of a particular protein in a mixture, but can also be used to study the antigen-antibody reaction.

The antibodies against a particular antigen are adsorbed to a solid support, in most cases a polystyrene microtiter plate.

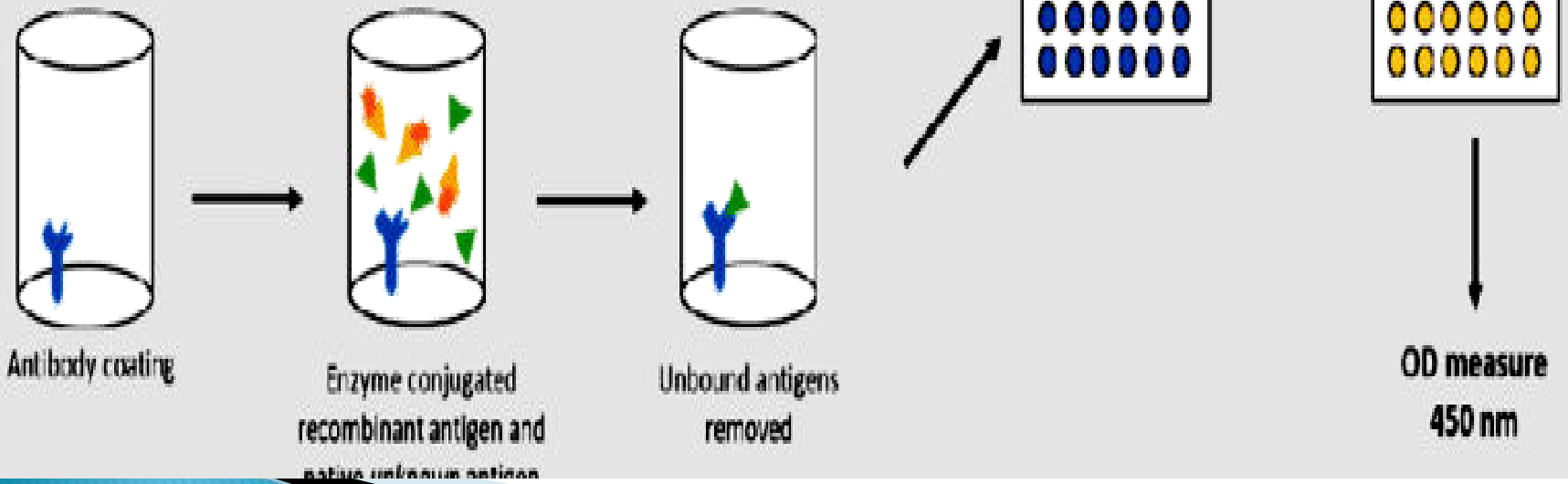
- The support after coating with antibody is washed and then the antigen is added, which binds to the adsorbed antibodies.
- An enzyme-linked antibody molecule called the conjugate is then added, which also binds to the antigen, which is followed by a chromogenic substrate for the enzyme.
- The colored product generated is observed for confirmation of antigen-antibody reaction as well as measured for quantitative estimation.
- The intensity of the color is proportional to the bound enzyme and thus to the amount of the bound antigen.


Capture Antibody
Detector Antibody
Detection Conjugate
Target Protein
Sample matrix Protein
Pre-coated micro-well plate
Add sample or standards, incubate
Sample and standards are removed, add detector antibody, incubate, wash
Add detection conjugate, incubate, wash
Add detection substrates, incubate, read at OD 450 nm

Indirect ELISA

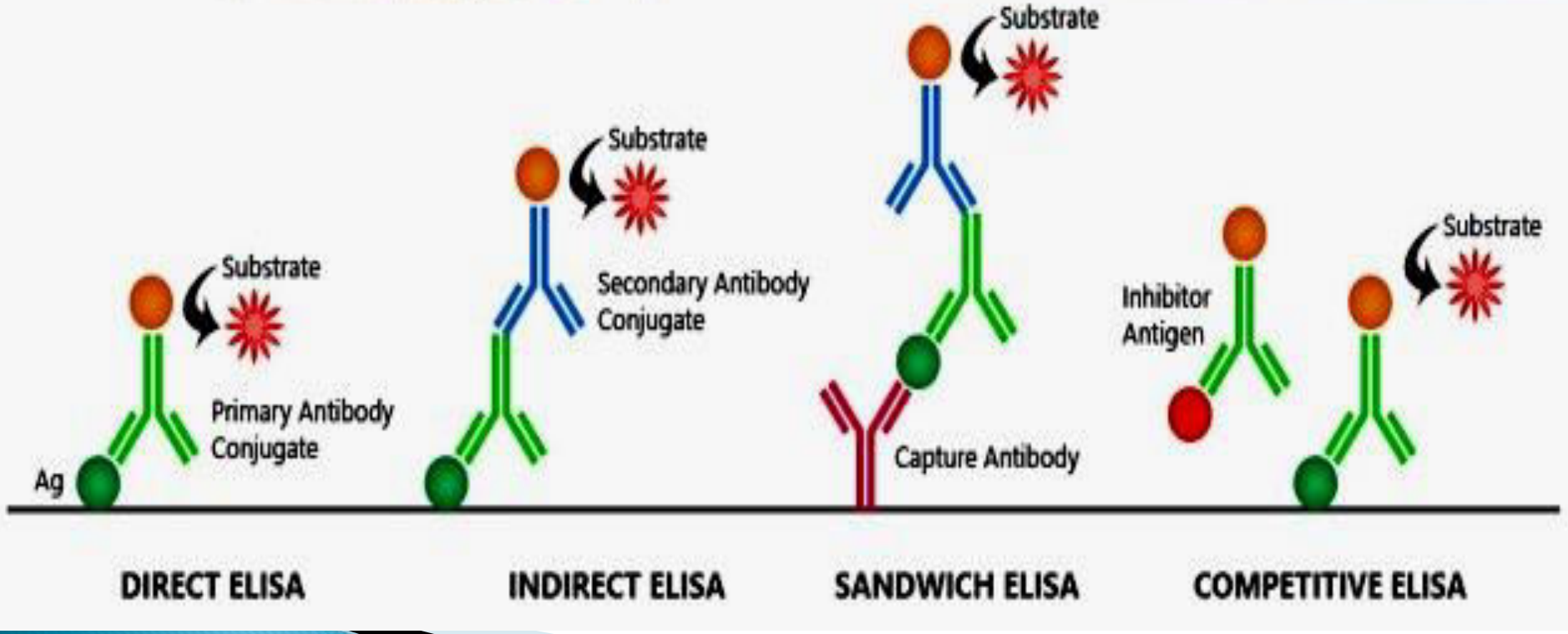


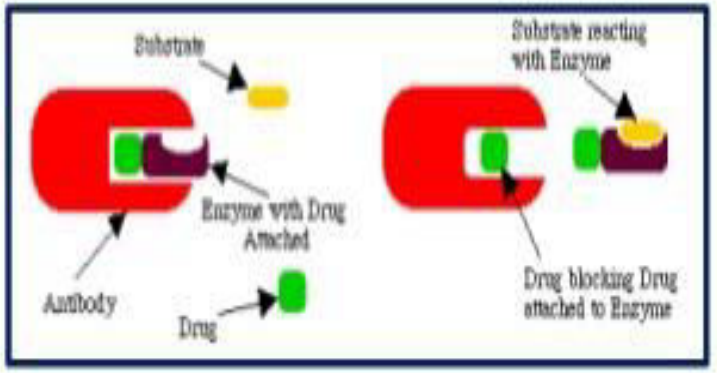
Competitive ELISA

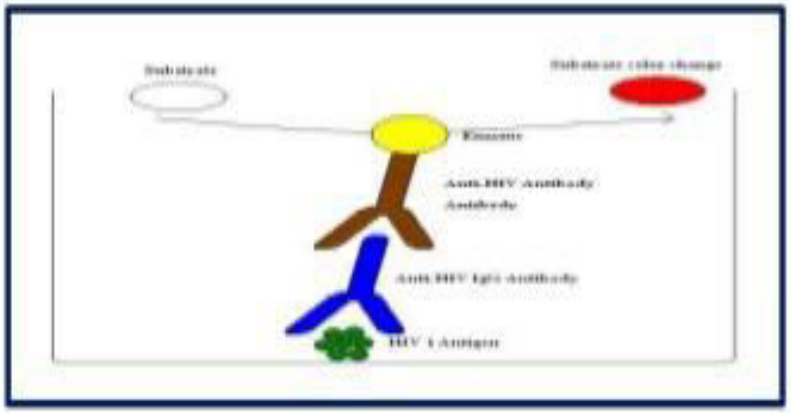


ELISA

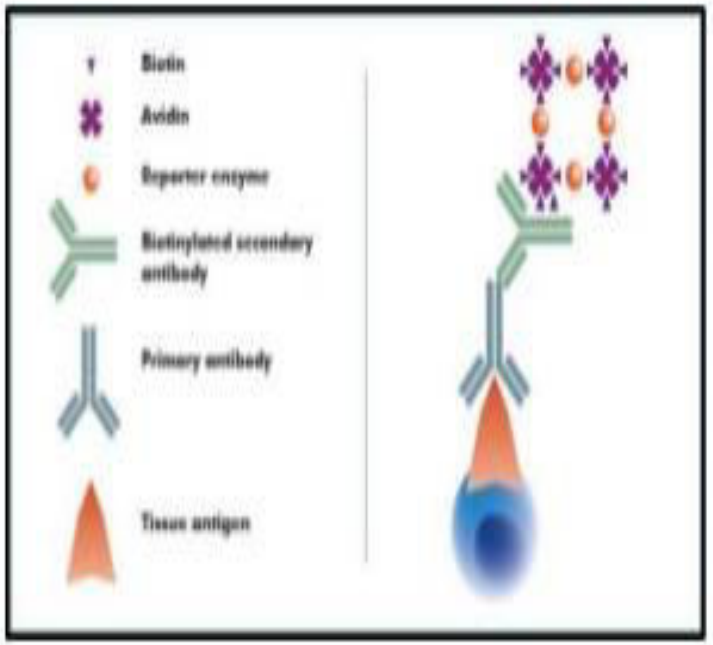
(enzyme-linked immunosorbent assay)







Enzyme Linked Immunosorbent Assay (ELISA) and its clinical applications



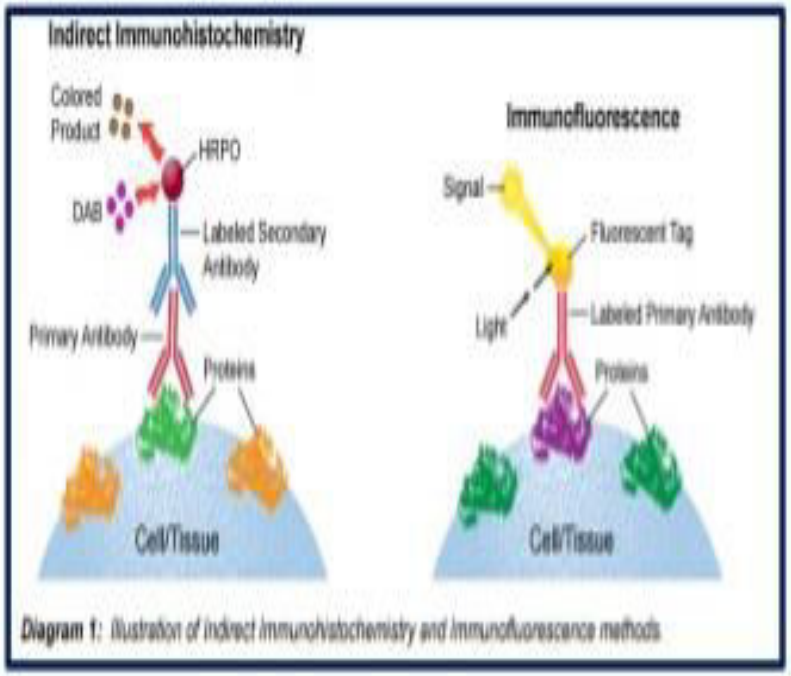
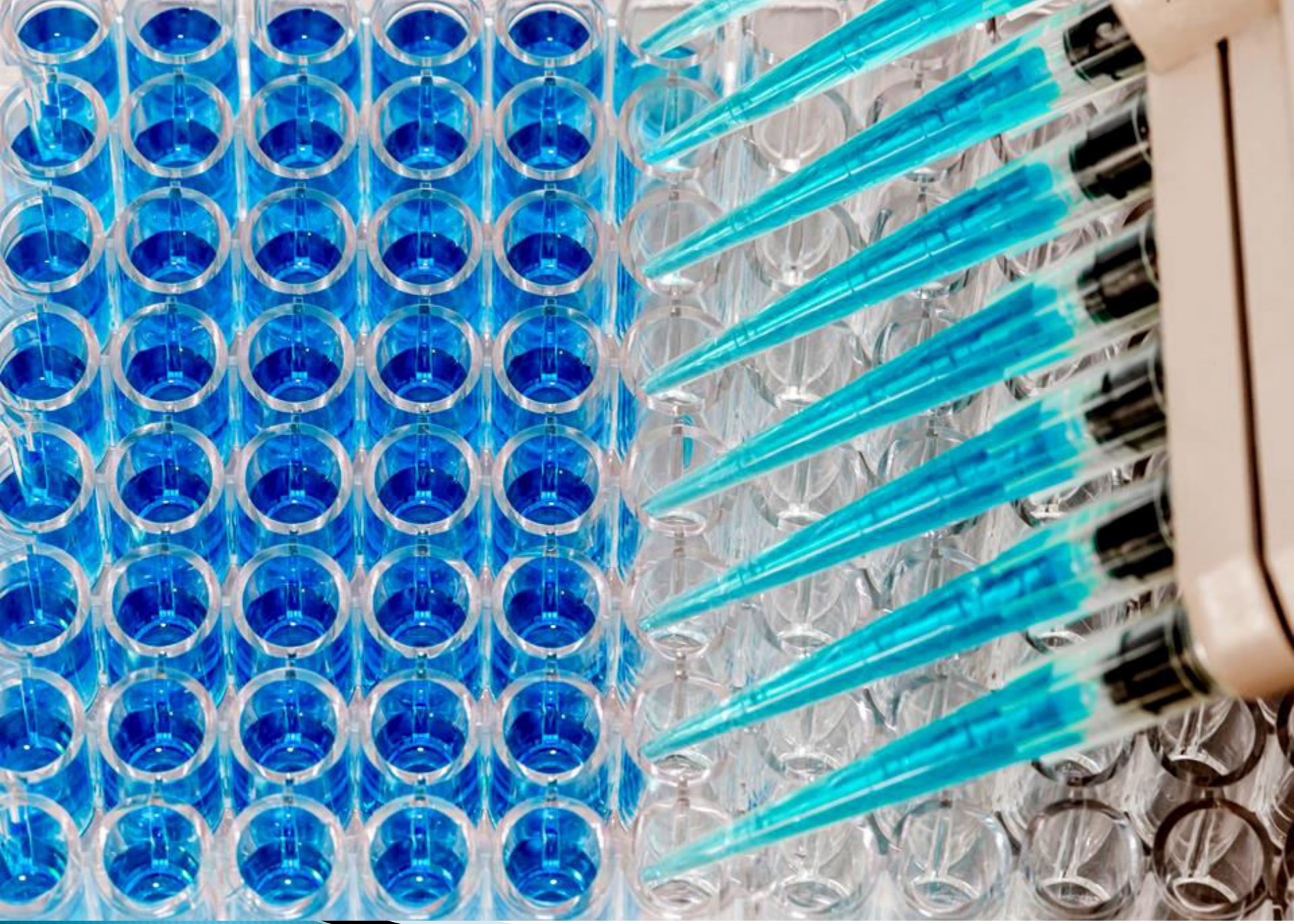


Diagram 1: Illustration of indirect immunohistochemistry and immunofluorescence methods

ELISA: An example of an assay using a 96-well plate.

The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.



3. Radio-Immuno Assay:

In this procedure, the antigens or antibodies are labelled with radioactivity, which facilitates their identification, even though they are in small concentrations.

Radioimmunoassay (RIA)

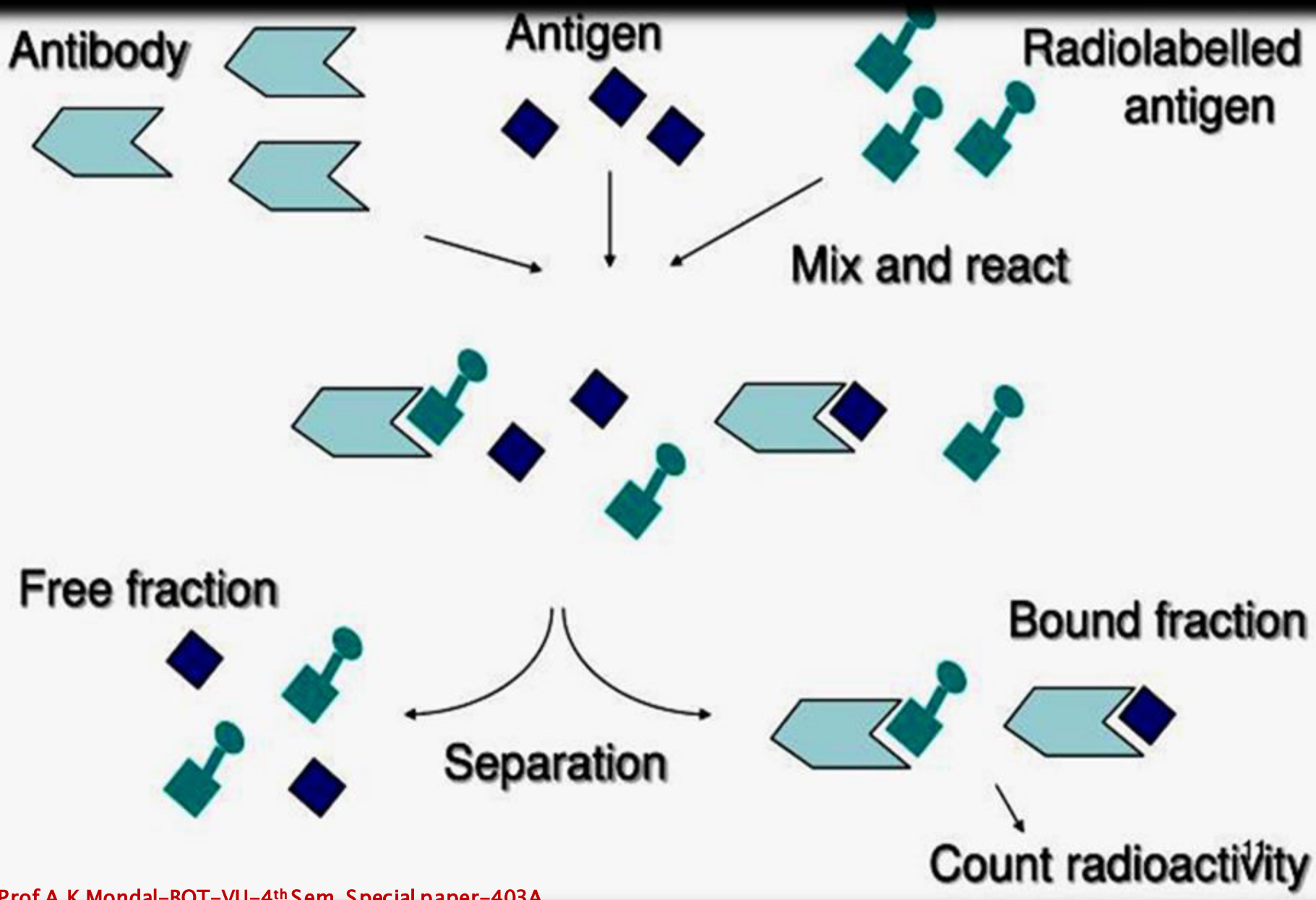
Radioimmunoassay (RIA) is an in vitro technique used to measure concentrations of antigens (for example, hormone levels in the blood) without the need to use a bioassay.

The technique was developed by Berson and Yalow in the year 1960 to measure concentration of insulin in the blood.

Principle of RIA

- Competitive binding of radiolabelled antigen and unlabelled antigen to a high affinity antibody.
- The labeled antigen is mixed with the antibody at a concentration that saturates the antigen-binding sites of the antibody.
- As the concentration of the unlabelled antigen increases more labeled antigen will be replaced from the binding site.

Molecular Mechanism of Radioimmunoassay (RIA)



SIGNIFICANCE AND LIMITATION OF SEROTAXONOMY:

- Serological studies have provided a correlation between serotaxonomy and the classical chemotaxonomic scheme in a number of instances.

- These studies mainly denote the relationship between different species and genera. However, they fail to specify the evolutionary relationships, because which antigen is the most primitive is unknown.

Role of Serotaxonomy in plant Systematics

Serological studies using crude plant protein extracts have been widely used in elucidating the taxonomy of a wide variety of higher-level taxa and in estimating phylogenetic relationships.

- Fairbrothers (1983) has used serological data in classification of orders and assignment of families in Apiales, Fagales, Magnoliales, Juglandales, Rubiales, Ranunculales etc.
- Fairbrothers and Jhonson (1959) has separated six species of Bromus on the basis of the serological data.

Examples

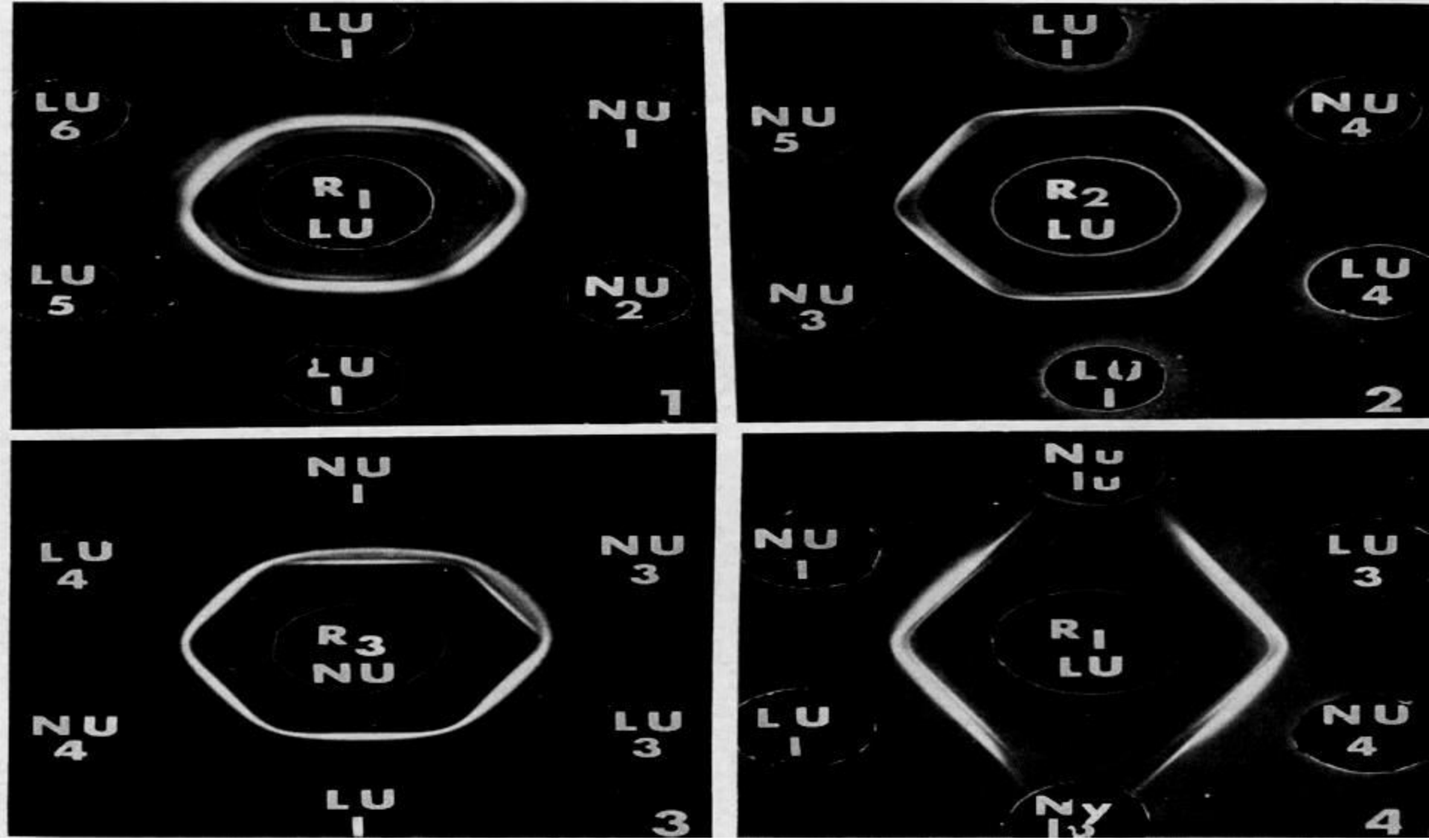
a. A close relationship among the Magnoliidae, Hamamelididae and Comiflorae of the angiosperms has been found, based on comparative serological studies of their major seed proteins. This has refuted the idea of their independent evolution.

b. The homogeneity of the iridoid-producing Comiflorae has been confirmed by serological studies, which has supported the inclusion of the Gentianaceae in it.

c. Based on phytoserological studies, Pickering and Fair brothers (1970) have proposed the classification of the family Umbelliferae into Hydrocotyloideae, Saniculoideae and Apioideae, and Apioideae was found to be more closely related to Saniculoideae than to Hydrocotyloideae.

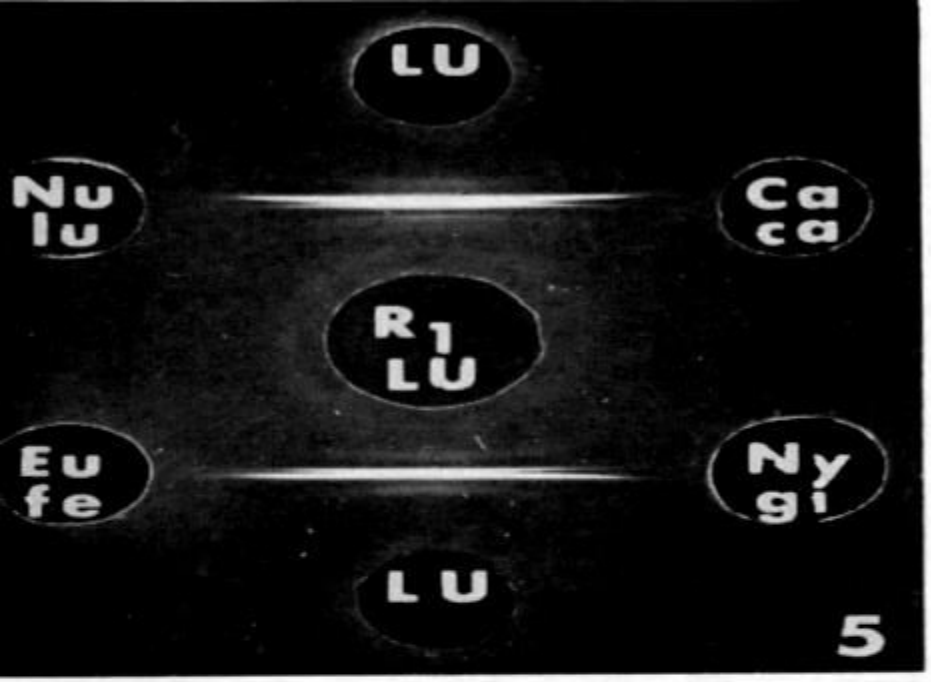
d. The Serological studies have shown that the genus Linodendron is quite distinct from other members of the family Magnoliaceae, and the genera Magnolia and Michelia are most closely related within the family.

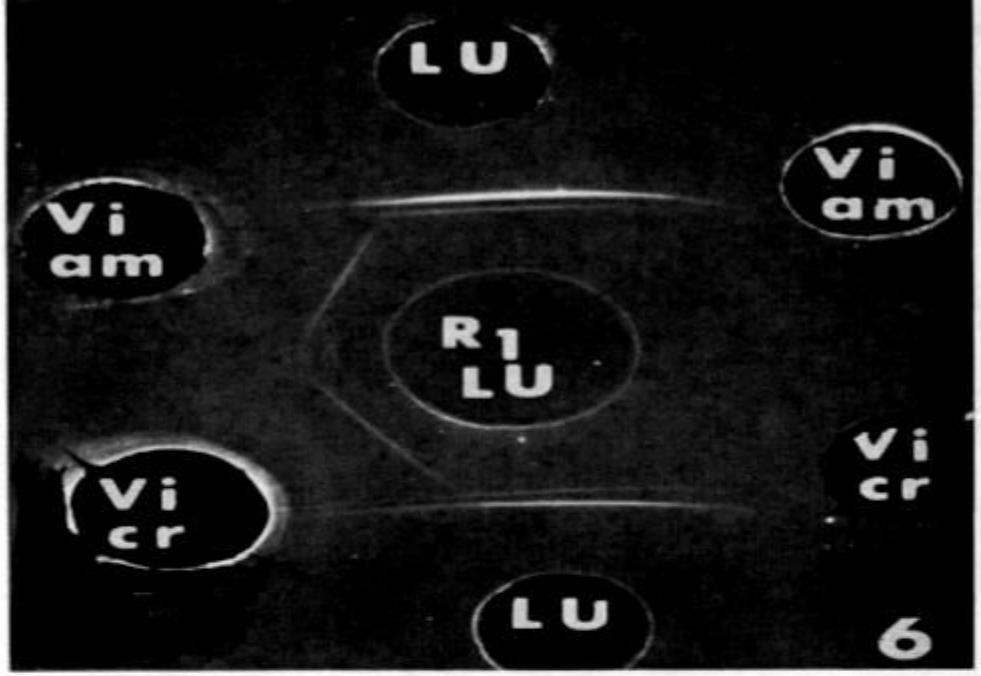
e. The Serological analysis has been useful in estimating the generic kinship in the Caprifoliaceae and the relationship between the Nymphaeaceae and Nelumbonaceae.

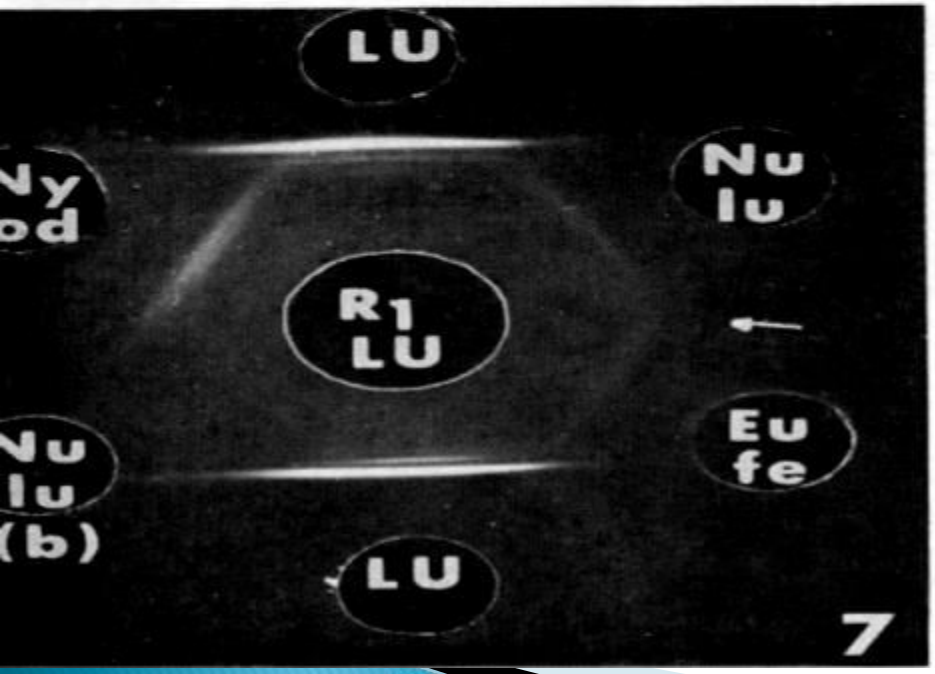


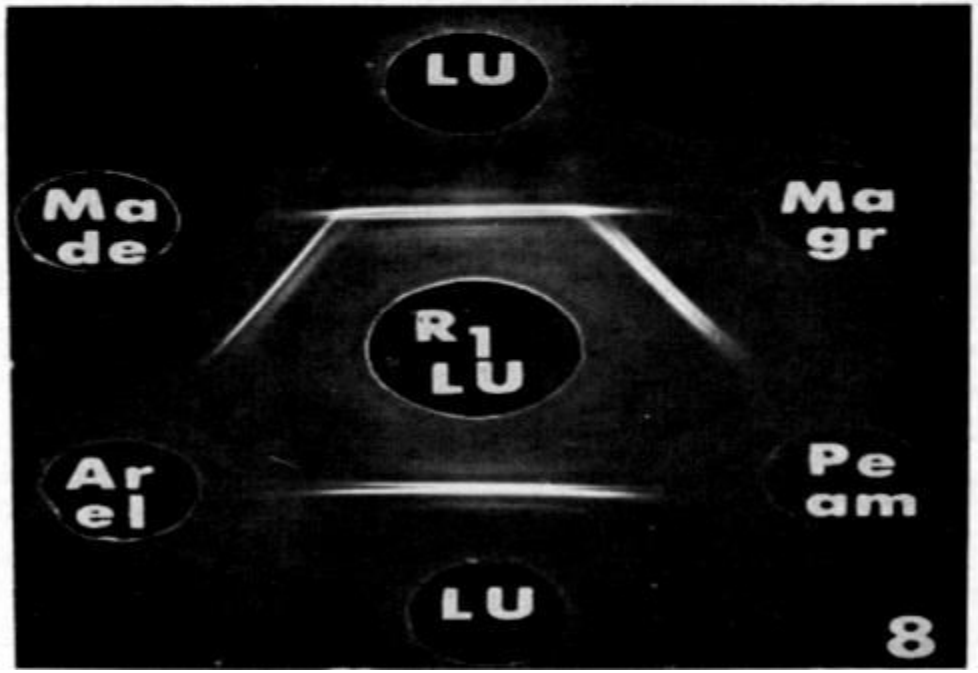
Immunodiffusion patterns produced in Ouchterlony plates from reactions between *anti-Nelumbo sera (R,, R,) or anti-N. nucifera senun (R,) and antigen extracts* of *Nelumbo and other Nymphaeales taxa.-KEY TO SYMBOLS: NU, antigen extracts of Nelumbo nucifera; LU, antigen extracts of N. lutea. Numbers following symbols refer* to accession analysed. Nu lu, *Nuphar luteum subsp . macrophyllum; Ny lo, Nymphaea lotus. (All extracts from nondelipified seed meals.)*

30th March, 2020


LU
Nu lu
Ca ca
R1 LU
Eu fe
Ny gl
LU
5


LU
Vi am
Vi am
R1 LU
Vi cr
Vi cr
LU
6


LU
Ny od
Nu lu
R1 LU
Nu lu (b)
Eu fe
LU
7


LU
Ma de
Ma gr
R1 LU
Ar el
Pe am
LU
8

f. Jensen (1968) discussed the relationships and classification of 20 genera of the Ranunculaceae based on serological evidence, which supports close relationship between Delphinium and Aconitum.

Hydrastatis, which is sometime classified with the Berberidaceae has more close serological similarity to the Ranunculaceae than the Berberidaceae.

Trollies, which is regarded as a link between follicular and achenial fruit bearing genera in the family, resembles closely with Adonis.

g. Lee and Fair brothers (1978) used serological techniques to study the systematics of Rubiaceae and related families.

The quantitative data emphasized the similarity of Rubiaceae to Cornaceae and Caprifoliaceae and the pre-saturation tests revealed similarity with Apocynaceae, Asclepiadaceae and Gentianaceae.

Similarly, two genera under Rubiaceae viz. Asperula and Galium were found to be serologically similar to each other while being the most dissimilar from all other genera of the family.

h. In an attempt to estimate generic interrelationships of the tribe Genisteae of the Leguminoseae, Cristofolini and Chiapella made a serological survey and found that the genus Cytisus (sensu lato), including Chamaecytisus, Sarothamnus and Carothamnus, is a single homogenous unit apart from C. sessilifolius, which in turn is more closely related to Laburnum and Genista than to other species of Cytisus.

Their studies also supported the elevation of the sect. Asterospartum of Genista into a new genus Cytisanthus.

j. A cross-reactivity serological reaction (i.e., the reaction between an antiserum and any antigenic material other than the antigenic material used in its formation) of the seed proteins of *Phaseolus vulgaris* with those of other species of the same genus by Kloz, showed a decreasing reaction in the series *P. coccineus, P. polyanthus, P. acutifolius, P. lanatus* and *P. aureus*, showing that, *P. vulgaris* is most closely related to *P. coccineus* and most distant to *P. aureus.*

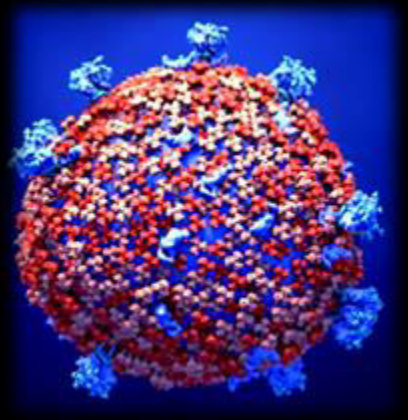
k. Gell, Hawkes and Wight (1960) and later Hawkes and Lester on the basis of double diffusion tests, compared potato species and showed that Mexican species fell in three groups and *Solanum morelliforme* is only distantly related to other tuber-bearing solanums.

Acknowledgements:

I would like to thank our *Honourable Vice Chancellor* Professor Ranjan Chakarborti for giving me the opportunity to contribute in E-learning process which will be very much helpful for our students during unprecedented situation due to CORONA Virus (COVID-19).

We shall overcome!!!!!!!!

#SAVE FROM CORONA



Stay Home

Save your Life

Save your Family

Save your Society

Save your Country

Save your beautiful Planet

THANK YOU