BIOLOGICAL TECHNIQUES

Prof. (Dr.) Amar P. GARG

Vice Chancellor

Shobhit Deemed University

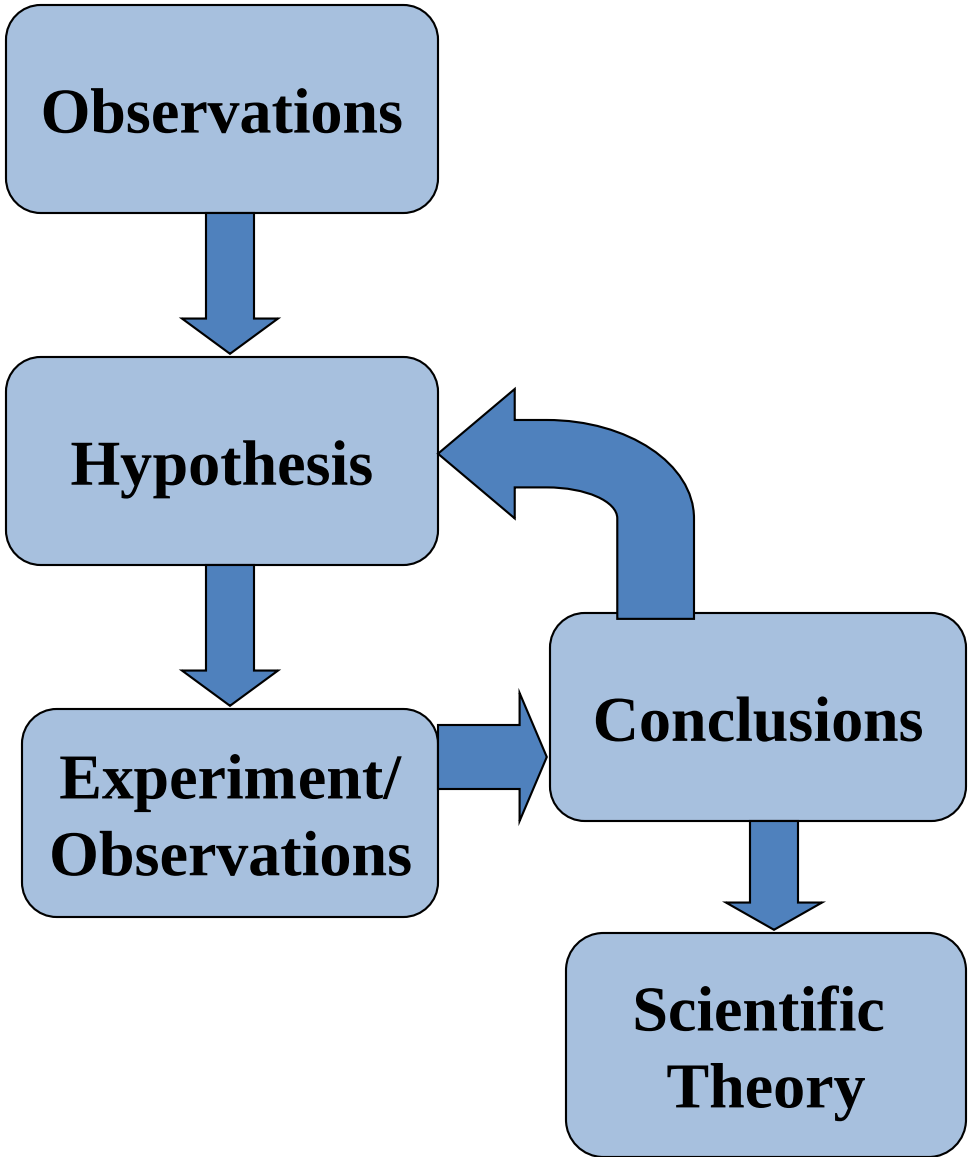
MODIPURAM

MEERUT-250110

E-mail: vicechancellor@shobhituniversity.ac.in

Scientific Method

This simplified flow diagram of the scientific method shows the important components involved in a scientific study.



Poor Knowledge on the Principles of Equipment Use

For example:

- **Spectrophotometers:**
  - **single and double wavelength,**
  - **Calibration,**
  - **use of glass and quartz cuvettes**
  - **Lambert-Beers law**
- **Calibration of balances, pipettes, bomb calorimeter and of other equipment**
- **Use of 'New Techniques' which are 'fashionable' without knowing the principles and their relevance**

Poor Knowledge on the Principles of Equipment Use…….. Contd.

- ***Separation using electrophoresis, Sephadex, and ion exchange resins***
- ***Preparation and storage of distilled water***
- ***Proper washing and storage of glassware***

Poor Knowledge on the Principles of Bioprocesses

For example:

1. ***How to measure enzyme activity? Linearity for time and concentration is rarely checked.***
2. ***How to define and express enzyme activity (IU/ml or IU/g)?***
3. ***Difference between enzyme activity and specific activity of enzymes.***
4. ***How buffer solutions work?***
5. ***Which buffers to use for which pH ranges?***
6. ***How to prepare buffers of different molar concentration?***
7. ***What is the difference between molar concentration (M), normality (N)?***
8. ***How to prepare standard acid/base solutions?***
9. ***How to prepare and use standard curve? How to make dilutions?***
10. ***Difference between rpm and g in centrifugation.***
11. ***Lack of basic rumen fermentation and tissue biochemistry.***
12. ***Similar principles associated with other techniques***

Poor Knowledge on the Use of Standards and Other Quality Control Principles

For example

- ***Standards rarely used for measurement of proteins, fibres and other chemical constituents.***
- ***Recovery of a known amount of chemical of interest rarely measured in the base material – effect of matrix is rarely investigated while using an assay system***
- ***Proper blanks and controls not used***
- ***Method validation of new or adapted methods rarely done.***
- ***Poor knowledge of techniques for isolation of microbes from different habitats***
- ***Little or no knowledge of identification of microbes from different sources.***

Poor Knowledge on Quality Assurance Procedures

- There is no place in good scientific research for the view that 'analysis is not worth spending time or money on'
- 'Rubbish in, rubbish out'

Improper General Laboratory Practices

- ***Poor record keeping.***
  - ***Generally loose sheets of papers used***
  - ***Ideas, plans, discussions, instructions, problems encountered rarely recorded***
- ***Proper protocols for collection of 'representative' samples from a bulk material, fluid or tissue not followed***
- ***Proper drying and storage conditions not used***
- ***Literature accompanying equipment/chemicals rarely referred to before use (or kept near the equipment)***
- ***Experimental glassware/items not properly labeled***
- ***Lack of critical thinking while recording data – a robotic approach used***

Lack of Information on Laboratory Safety Issues

For example:

- Laboratory safety issues most neglected
- Difference between bio-safety and bio-security
- Safe discard of hazardous chemicals
- Safe discard of pathogenic microbes
- Safety issues in case of any accidental contact with biologically/chemically dangerous object
- Common use of eatable in the laboratory
- No proper earthing of electrical circuits in the lab and no constant and continued voltage
- No proper labeling of chemicals, no proper and safe storage of hazardous chemicals

Improper Presentation of Results

- *While reporting a measured value, uncertainty (a measure of error associated with the value) is rarely given*
- *Data not properly presented, should be in understandable form*
- *Improper presentation of Tables and Figures with misleading titles and without captions on 'X' and 'y' axis.*
- *Use of improper statistical test and improper interpretation of data*
- *Use of improper decimal points in Tables*
- *No mention of Tables and Figure numbers in the Results and Discussion*
- *Discussion of results poor*

CENTRIFUGATION

Centrifuges

- Are instruments used to separate cells or cell organelles using centrifugal force.
  - Ordinary table-top centrifuges are used in cell cultures to isolate whole cells from culture media.
  - High-speed centrifuges or ultracentrifuges are used to isolate different shapes and sizes settle at the bottom of a centrifuge tube at different sedimentation rates. These are capable of spinning up to 75 000 revolutions per minute (rpm), producing forces equivalent to around 500 000 times that of Earth's gravity.

Chromatography

- It refers to a variety of techniques used to purify biological molecules, such as proteins and nucleic acids.
- A substance to be purified is suspended in a liquid medium (mobile phase) and is passed on to a column of matrix, such as beads (immobile phase). The substance to be purified interacts with the matrix and the interaction is used as a basis of separation.

Ion Exchange Chromatography

- Ionic charge of a substance is used as the basis for purification.

Gel Filtration Chromatography

- Makes use of the size of the molecule as the basis of purification.

Affinity Chromatography

- Uses very special and very specific interaction between two molecules.

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

Gas Electrophoresis

- is a powerful technique used to separate and visualize proteins or nucleic acids. Substances to be analyzed are driven along a gel of cross- linked molecular sieves using an electric current.
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Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

-It is therefore used to analyze the molecular weight of a given protein.

-is used to analyze proteins based on its molecular mass. It is therefore used to analyze the molecular weight of a given protein.

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

TYPES OF ELECTROPHORESIS:

ISOELECTRIC FOCUSSING

- is a type of electrophoresis that separates proteins according to isoelectric pH.

Two-dimensional gel electrophoresis

- An electrophoresis that combines both SDS-PAGE and isoelectric focusing

Agarose gel electrophoresis

- is used to analyze and determine the molecular weights of nucleic acids, such as DNA or RNA

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

Spectrophotometry

- Is an instrument used to determine the concentration of proteins or nucleic acids in a solution.
- Used in bacterial cell cultures to estimate the amount of cells present in a given volume of cell culture medium.
- Measure the amount of light at a specific wavelength that is absorbed by the solution, which is proportional to the concentration of substances present in the solution or the number of cells in a medium.

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

Polymerase Chain Reaction (PCR)

- Is a method used to amplify or make copies of a given DNA fragment using an enzyme called DNA polymerase.
- Based on a principle that a double-stranded DNA molecule breaks into two individual strands at high temperatures and with the use of PCR primers (short DNA strands), the DNA polymerase can synthesize two double-stranded DNA using two separated individual strands as templates. When the process is repeated over and over again, there is an exponential increase in the number of double- stranded DNA that is produced: from one copy to two copies to four to eight to sixteen to thirty-two, and so on, depending on how many times the cycle is repeated.
- Widely used as a tool in DNA cloning, analysis of genetic diseases, forensics, legal cases such as paternity testing and many more.

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

DNA Sequencing

- Is used to determine the sequence of nucleic acids present in a given gene or DNA fragment.
- This technology was independently developed by Fredrick Sanger and Walter Gilbert.
- Most automated DNA sequencers used today are developed by Sanger.
- Used to prepare the DNA sample to be sequenced, followed by gel electrophoresis. The results are tabulated and analyzed by a computer.

MODERN BIOLOGICAL TOOLS AND TECHNIQUES

Immunoassays

- Refer to a wide variety of techniques that use antibodies to recognize a very specific substance called antigen, such as protein.
- Widely used in the development of diagnostic kits used in hospitals to identify a particular disease or the presence of bacterial and viral infections.
- The pregnancy test kit is an example of an immunoassay

Western blot analysis

- A type of immunoassay used to confirm the identity of a protein immobilized into a membrane.

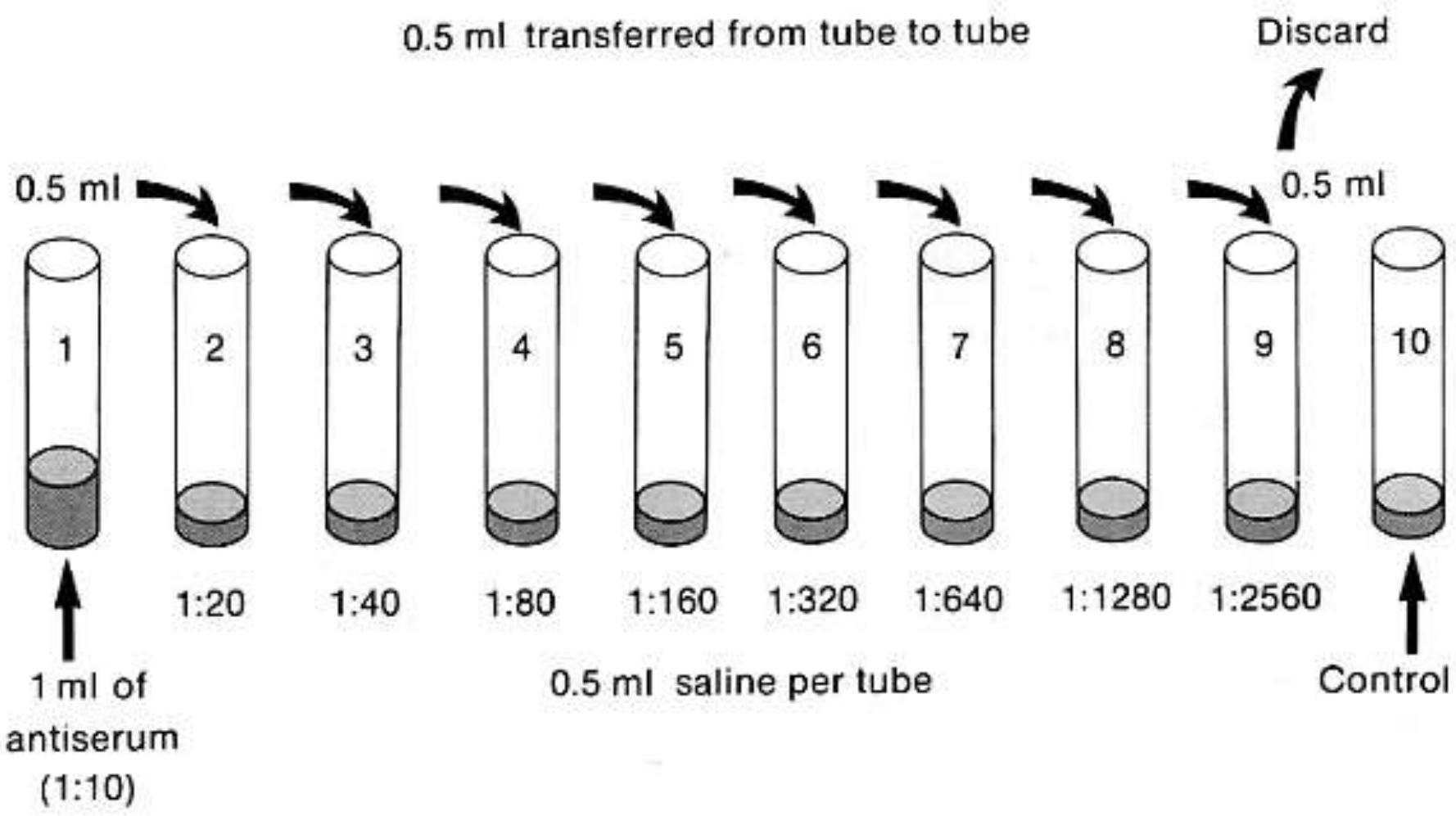
Enzyme-linked immunosorbent assay (ELISA)

- Detects proteins or antibodies bound on a plastic plate (ELISA plate) in a liquid system.

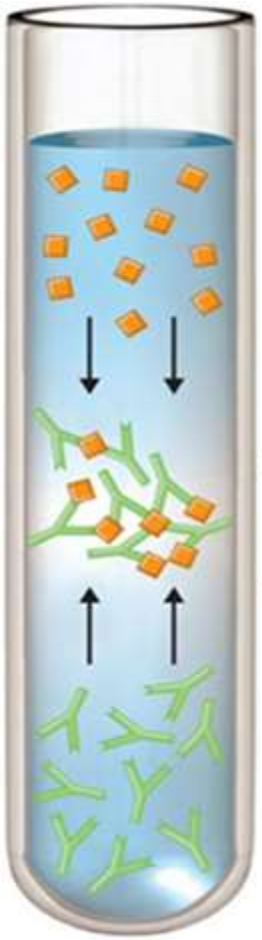
Immunofluorescence microscopy

- Used to identify the location of certain organelles or proteins inside a cell

Basic Principle of Dilution



Precipitation Reaction

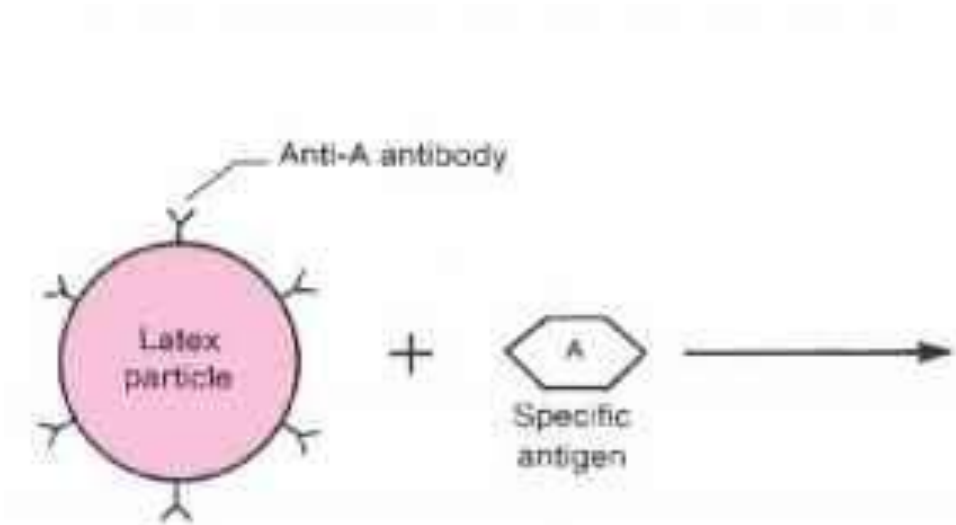


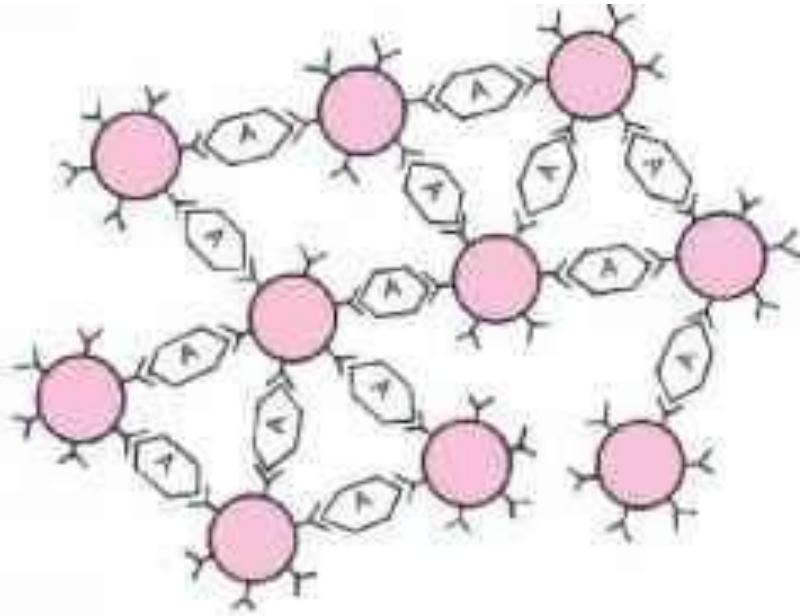
Antigens
(soluble)

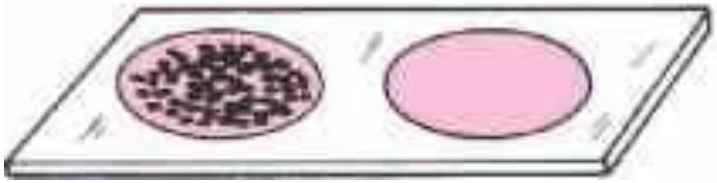
Zone of equivalence:
visible precipitate

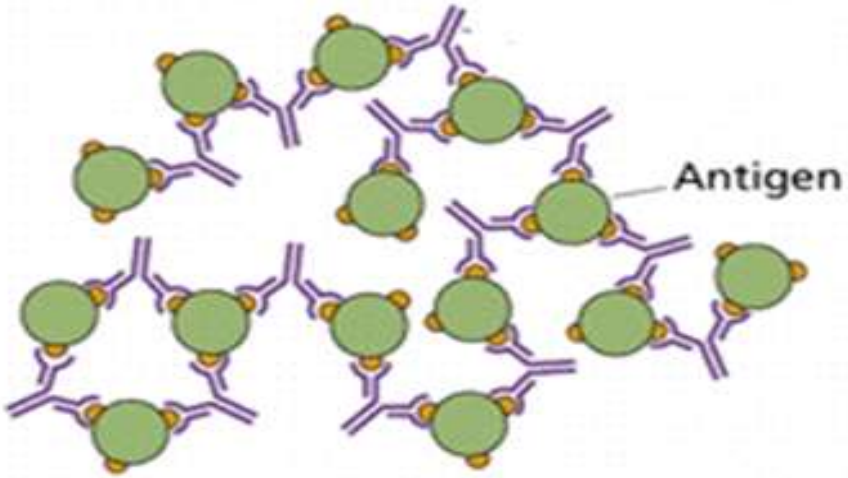
Antibodies

Agglutination Reaction

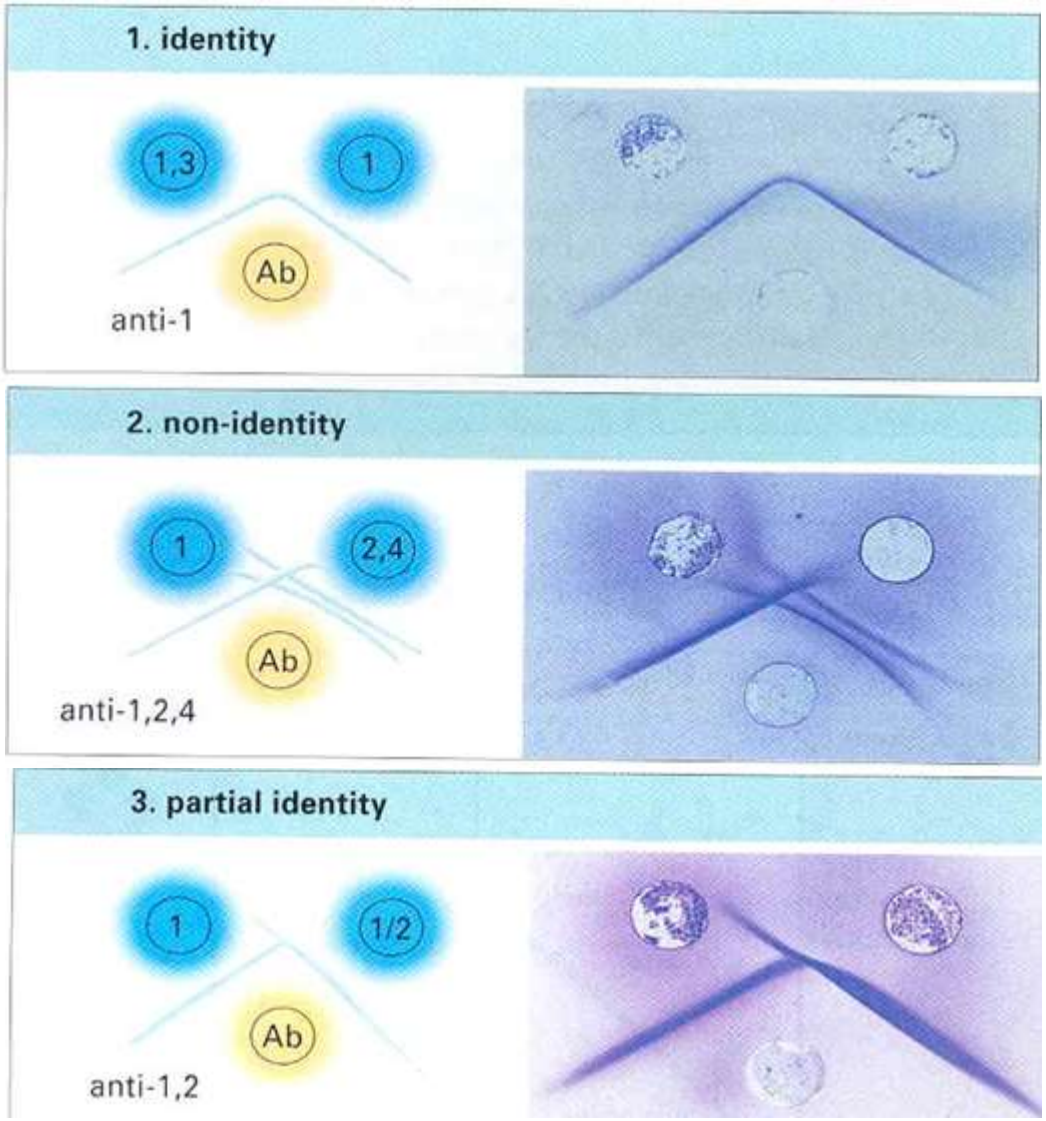


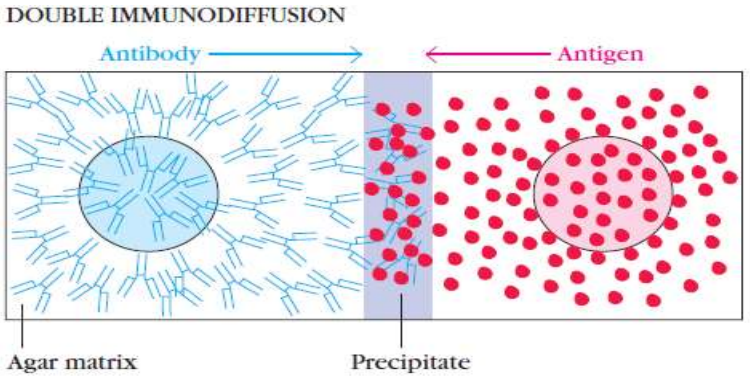


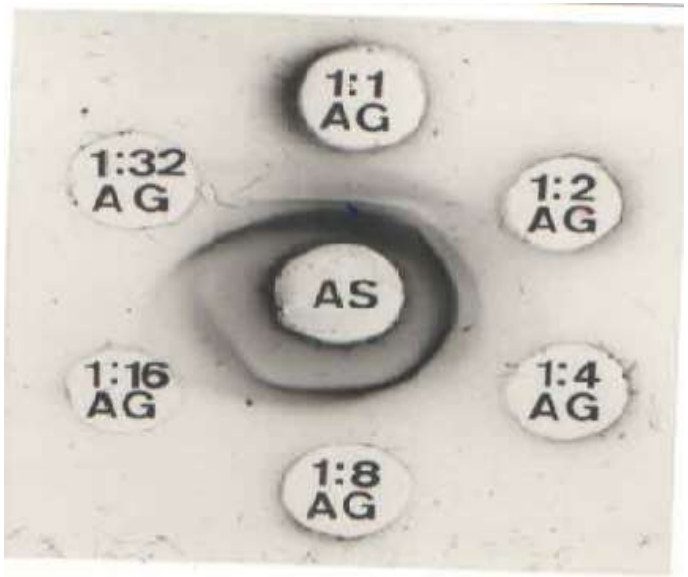




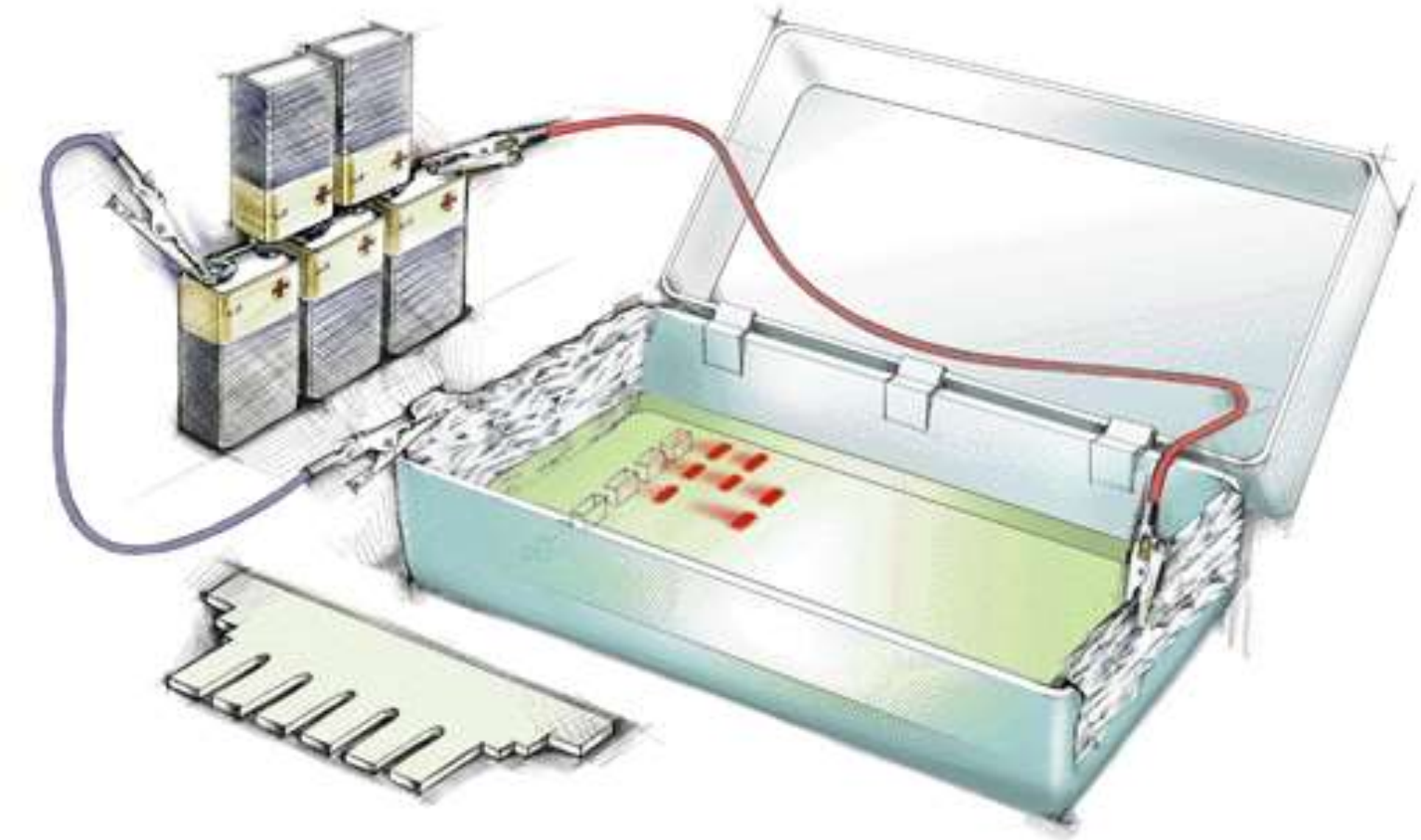
Ochterlony Immuno-double Diffusion



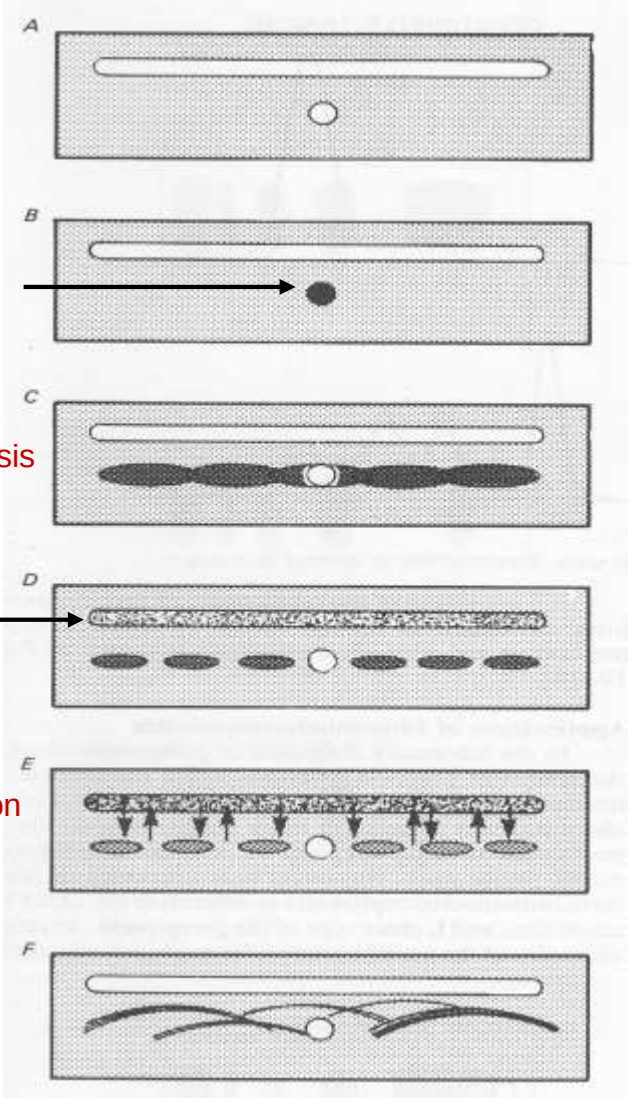




Principle of Electrophoresis

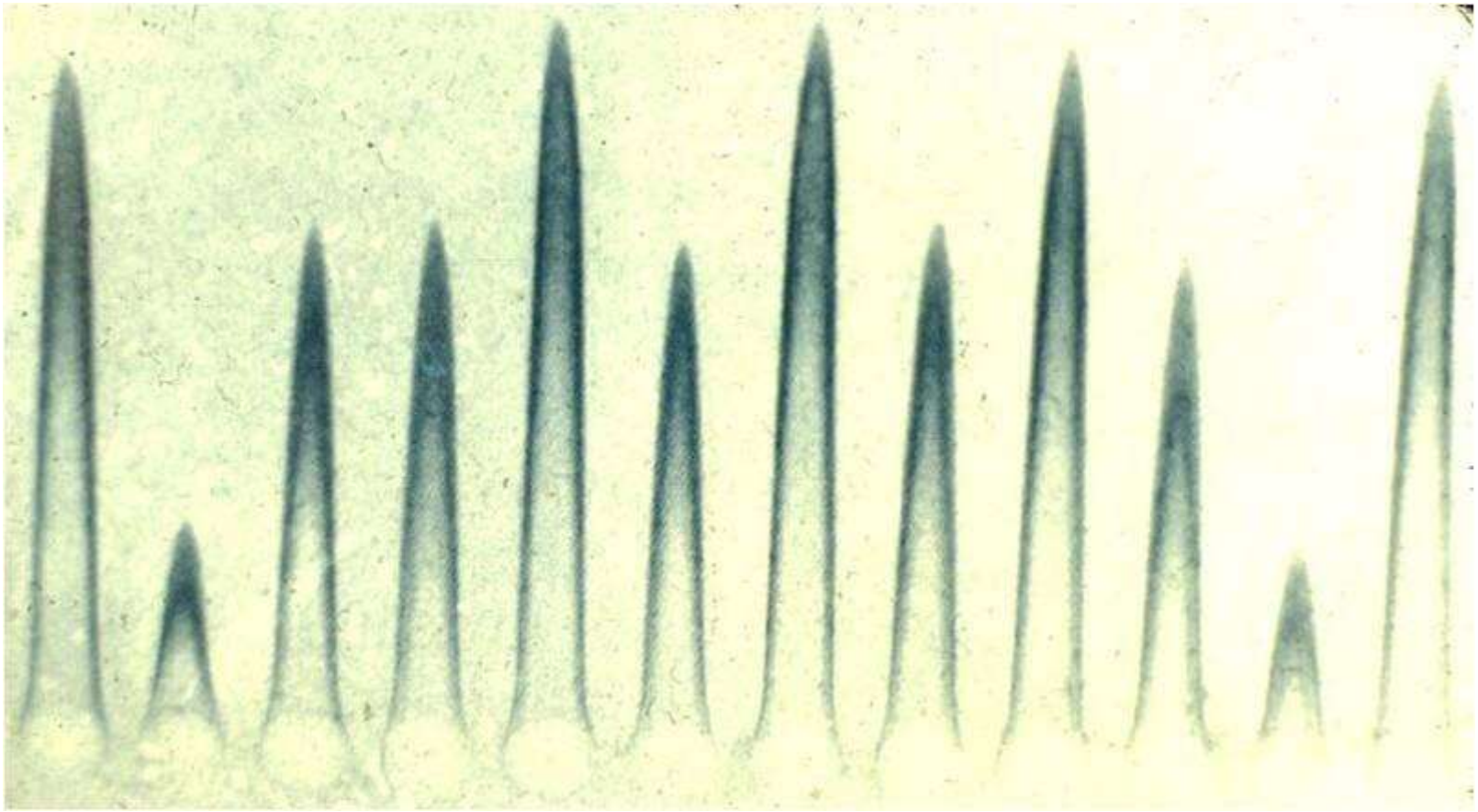


Immunoelectrophoresis



It combines electrophoresis separation, diffusion and precipitation of protein antigens

Rocket Immunoelectrophoresis

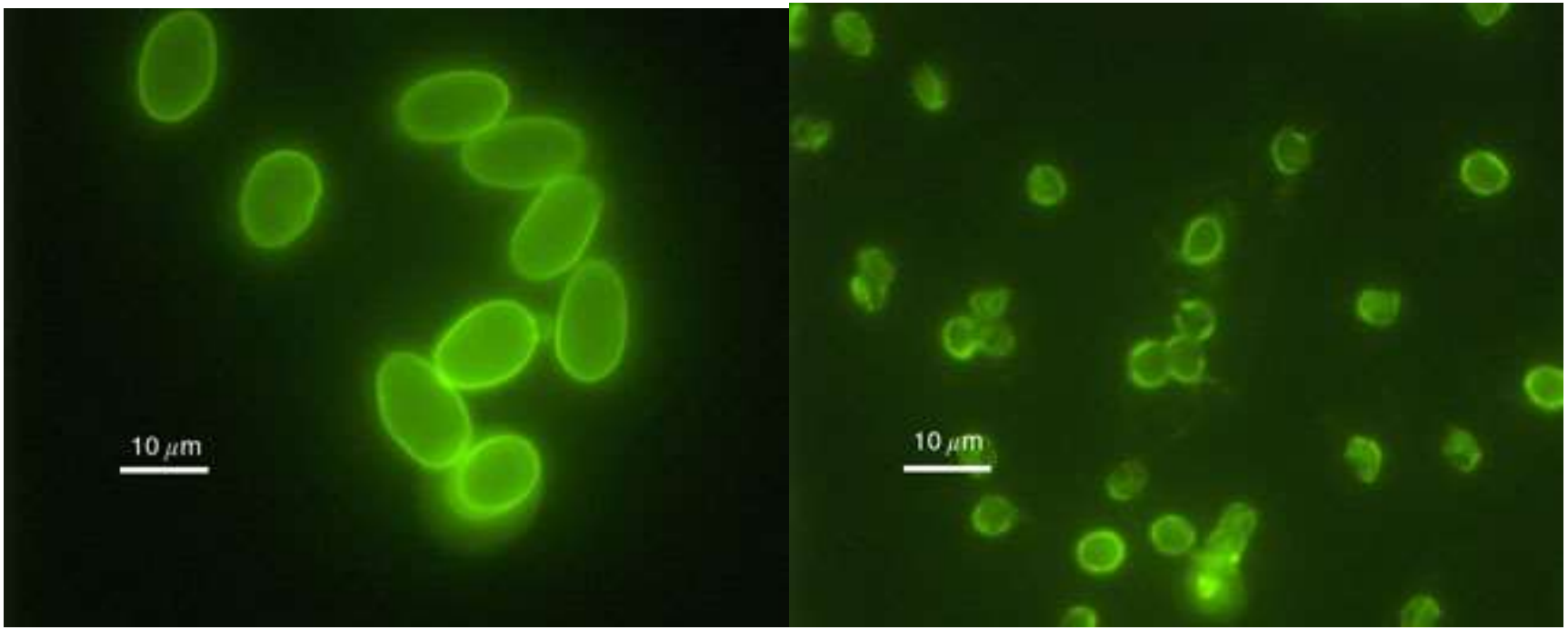


Crossed Immunoelectrophoresis

Secondary Antibodies Production

- ***Enhance sensitivity of immuno-reactions***
- ***When antibodies of one animal are injected into another/second animal, they act as antigens in second animal***
- ***Antibodies produced in second animal are called "secondary antibodies"***
- ***Purified secondary antibodies are labeled with horse radish peroxidase enzyme / gold particles / ferritin molecules / fluorescent dyes***

Immunofluorescence

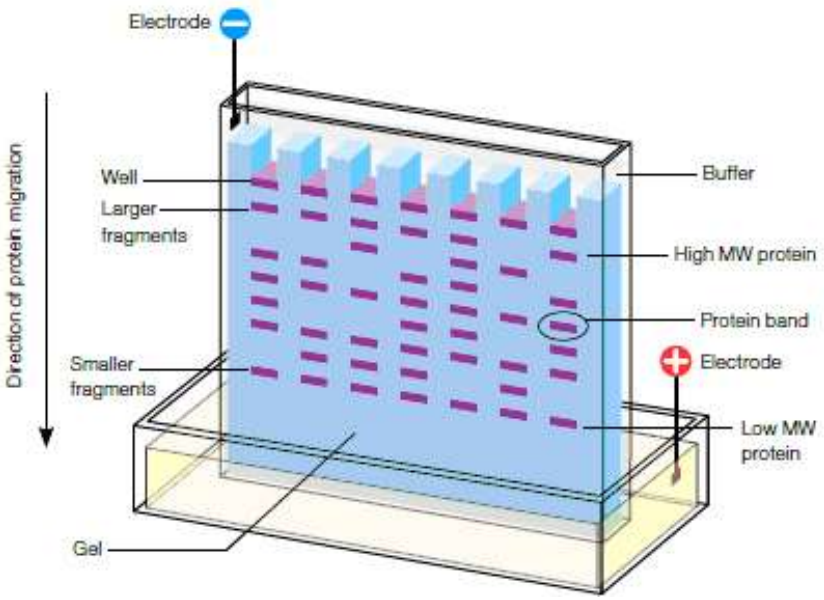


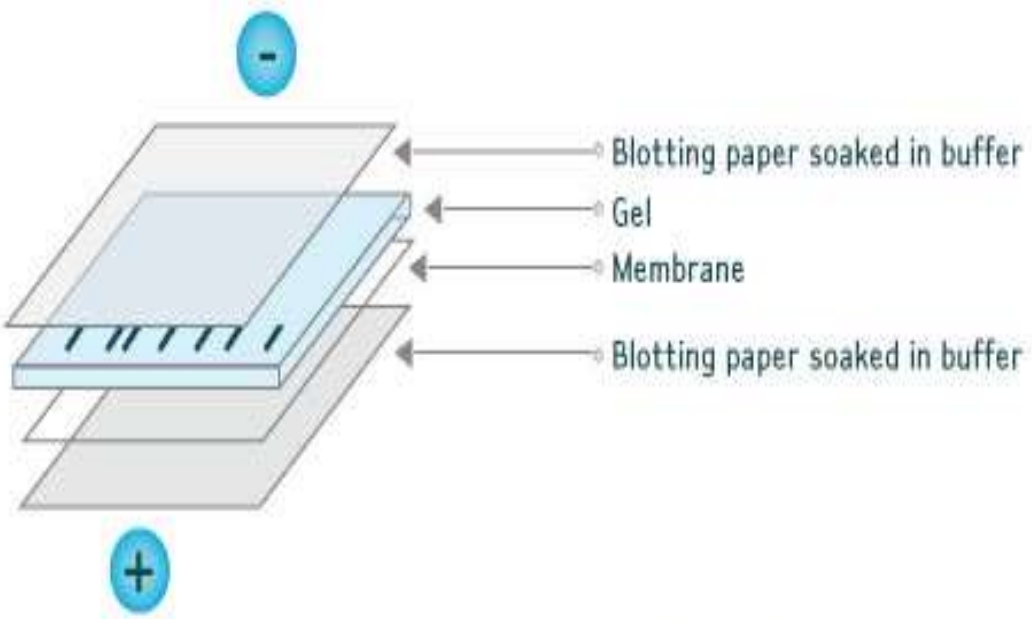
Fluorescent Antibody Staining
***Giardia* (left)**
***Cryptosporidium* (right)**

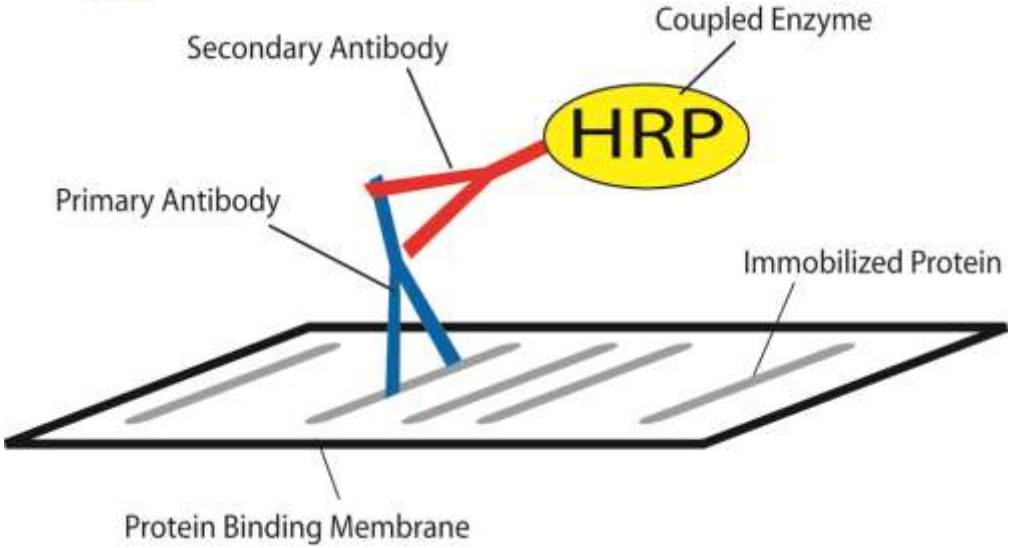
Immunoblotting

- ***Proteins are separated on SDS-PAGE***
- ***Immobilized on Nitrocellulose membrane***
- ***Membrane is cut in two pieces-one containing marker + proteins; another only protein bands***
- ***Additional sites blocked with 2% skimmed milk***
- ***Second piece cut into 5 mm wide stripes,***
- ***Reaction allowed with primary Ab, washed with PBS + Tween-20 followed by reaction with secondary Ab, chromogen substrate added and reaction developed with H_2O_2.***
- ***Bands observed, molecular wt. determined***

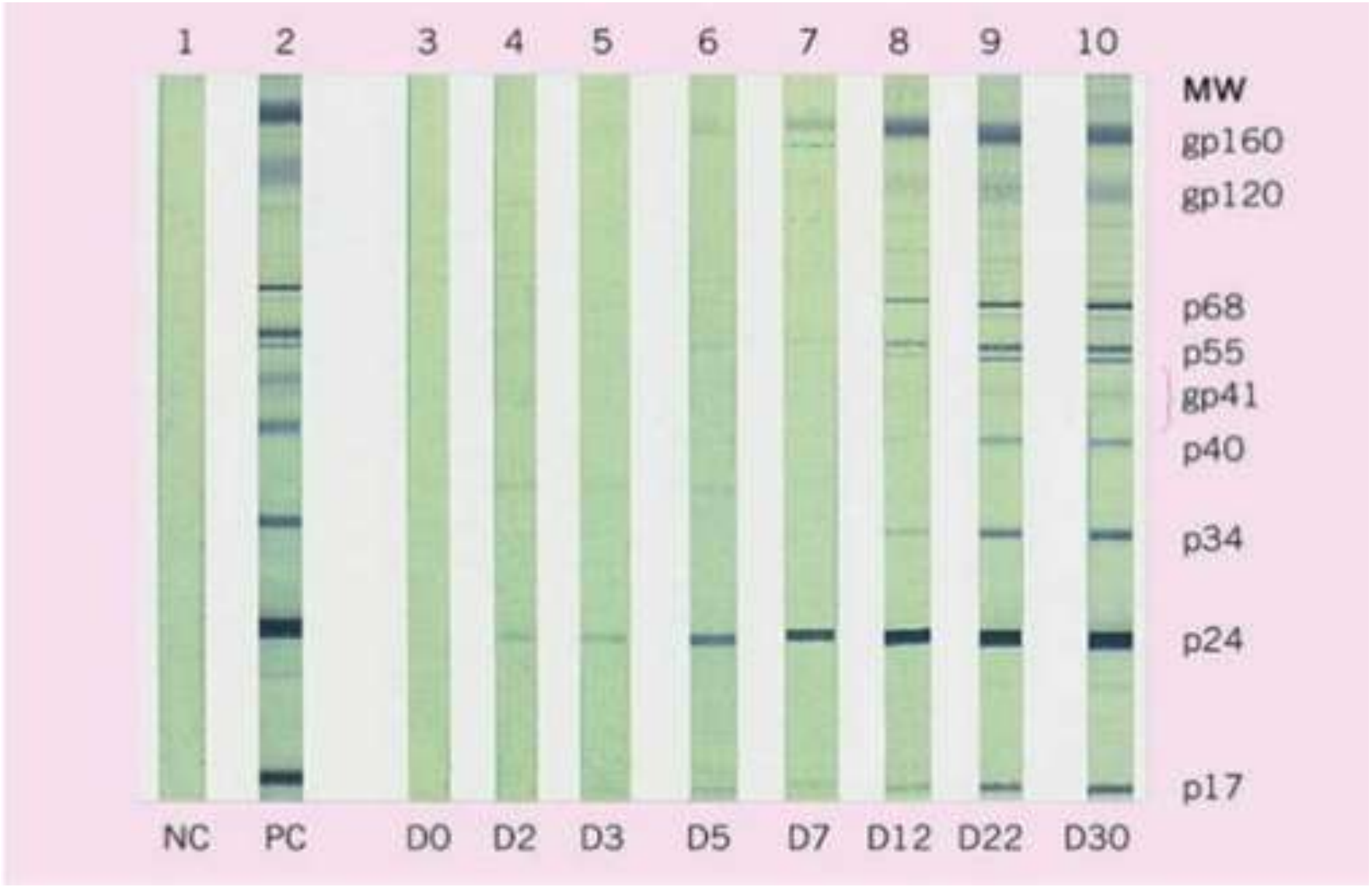
Major Steps in Immunoblotting







Typical Western Blot of HIV patients



Immunoblot-when proteins are not dominant antigens

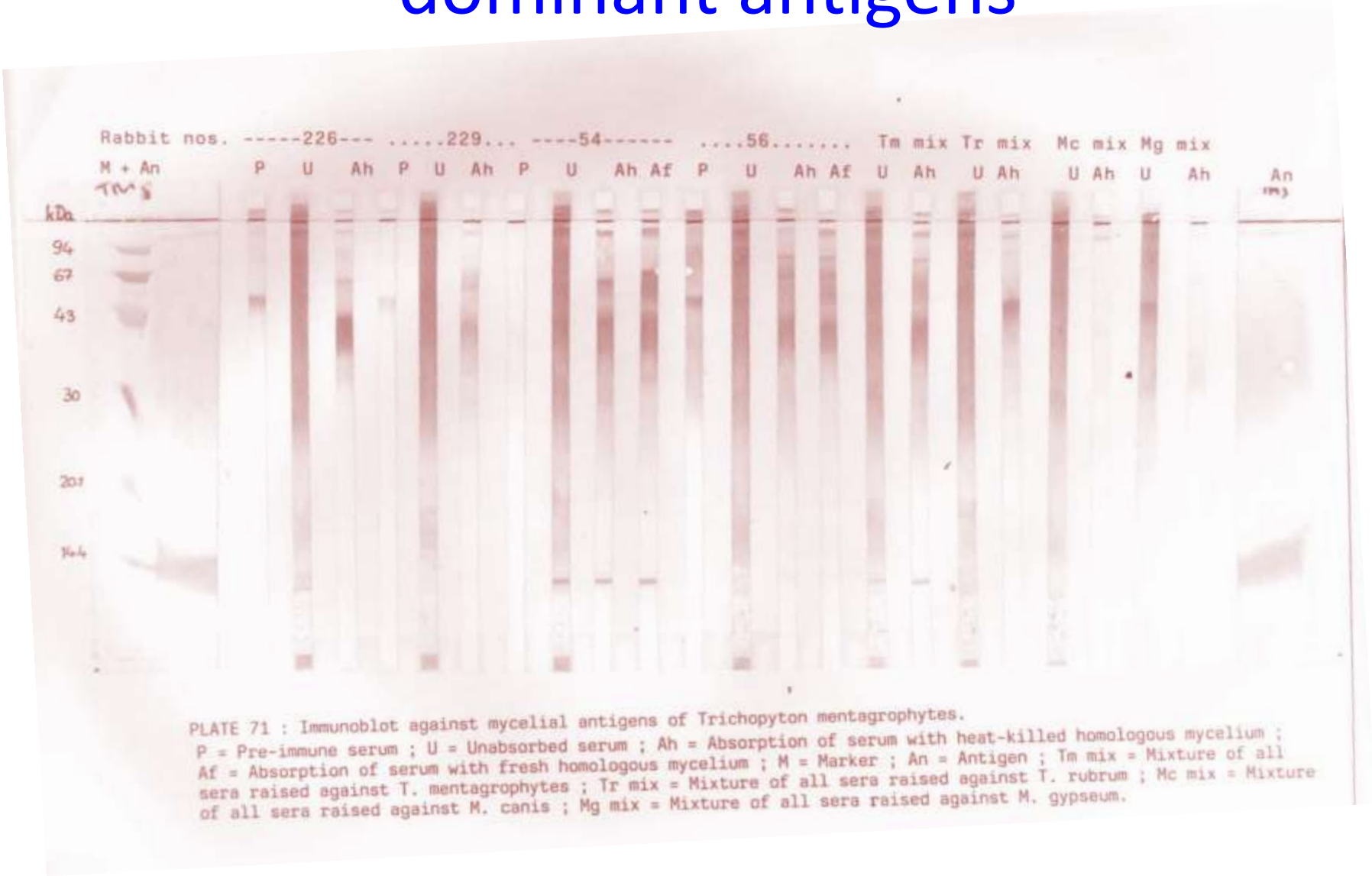
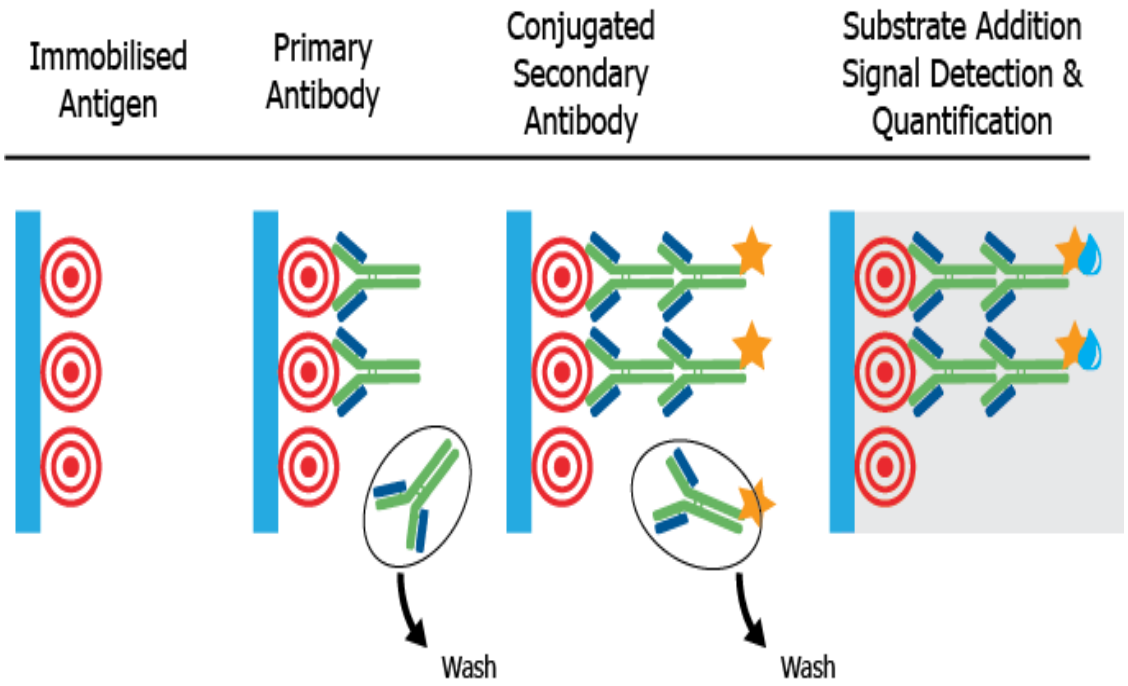


PLATE 71 : Immunoblot against mycelial antigens of Trichopyton mentagrophytes. P = Pre-immune serum ; U = Unabsorbed serum ; Ah = Absorption of serum with heat-killed homologous mycelium ; Af = Absorption of serum with fresh homologous mycelium ; M = Marker ; An = Antigen ; Tm mix = Mixture of all sera raised against T. mentagrophytes ; Tr mix = Mixture of all sera raised against T. rubrum ; Mc mix = Mixture of all sera raised against M. canis ; Mg mix = Mixture of all sera raised against M. gypseum.

ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA)



RIA and DOT BLOT are the variations of ELISA and Immunoblot respectively.

IMMUNO-ELECTRON MICROSCOPY

- ***PRE-EMBEDDING*: Cells treated with primary antibodies, washed, treated with secondary antibodies labeled with gold particles or ferritin molecules**
- ***POST-EMBEDDING*: Ultra thin sections allowed to react with primary antibodies followed by washing, reacted with secondary antibodies labeled with gold particles or ferritin molecules**
- **Normal Electron Microscopy followed**

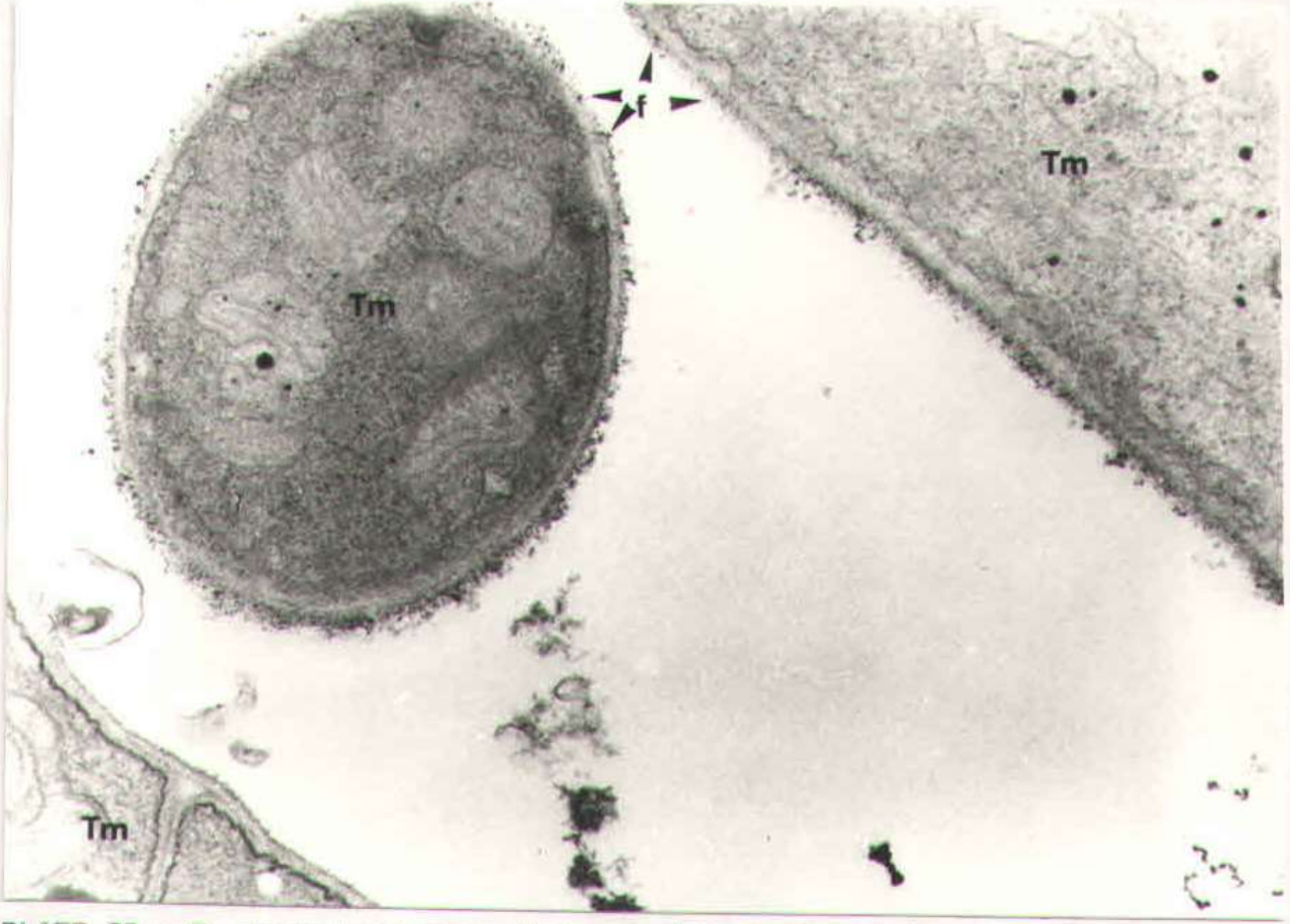


PLATE 83 : T. mentagrophytes, Untreated Control, I. Absorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F ,´Moderate Ig-binding , Cells are healthy (O.M. 10000 x, F.M. 26500 x, 23696).

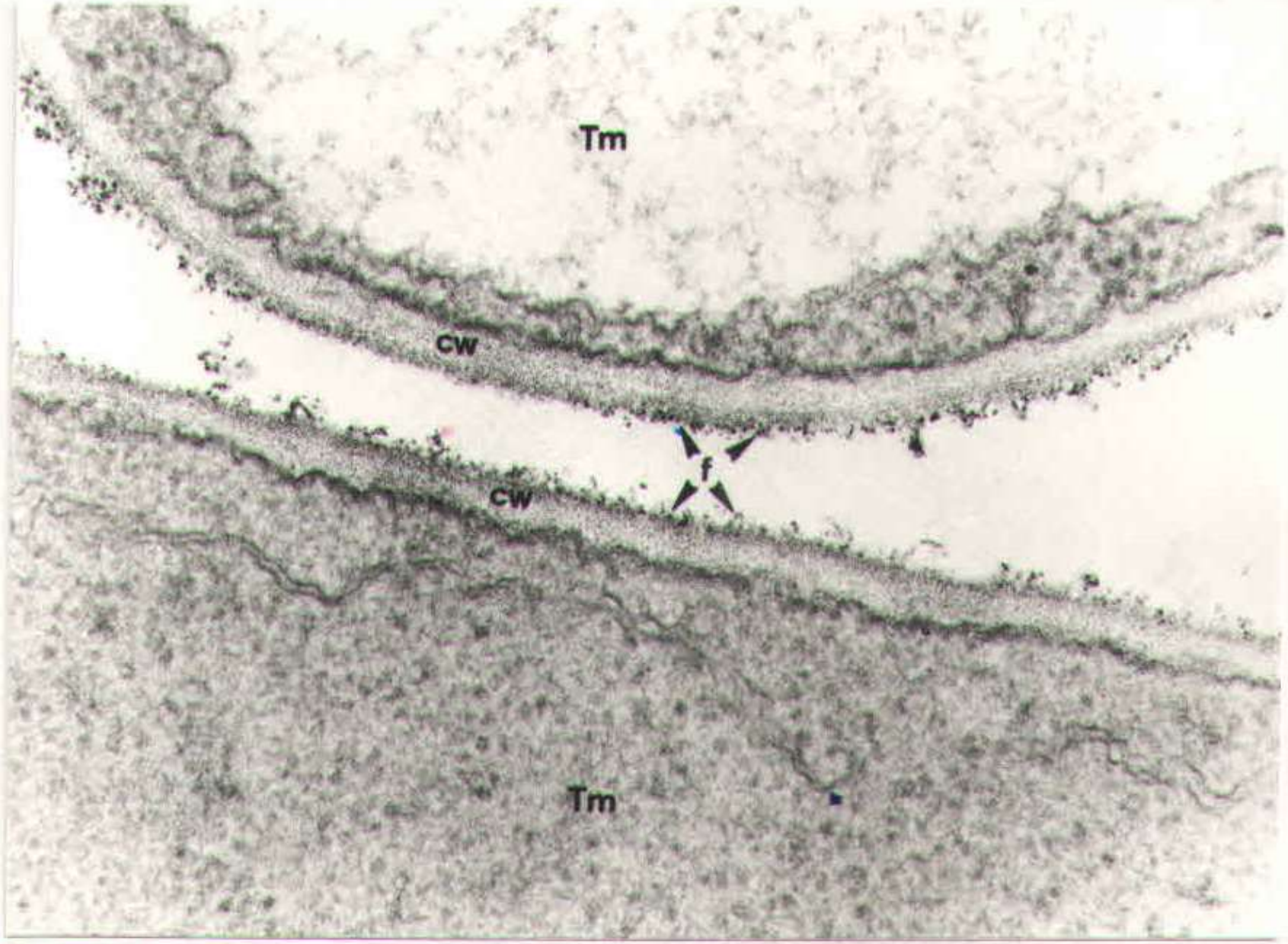


PLATE 84 : T. mentagrophytes, Untreated Control, I. Absorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F , Moderate Ig-binding , Cells are healthy (O.M. 30000 x, F.M. 80800 x, 23692).

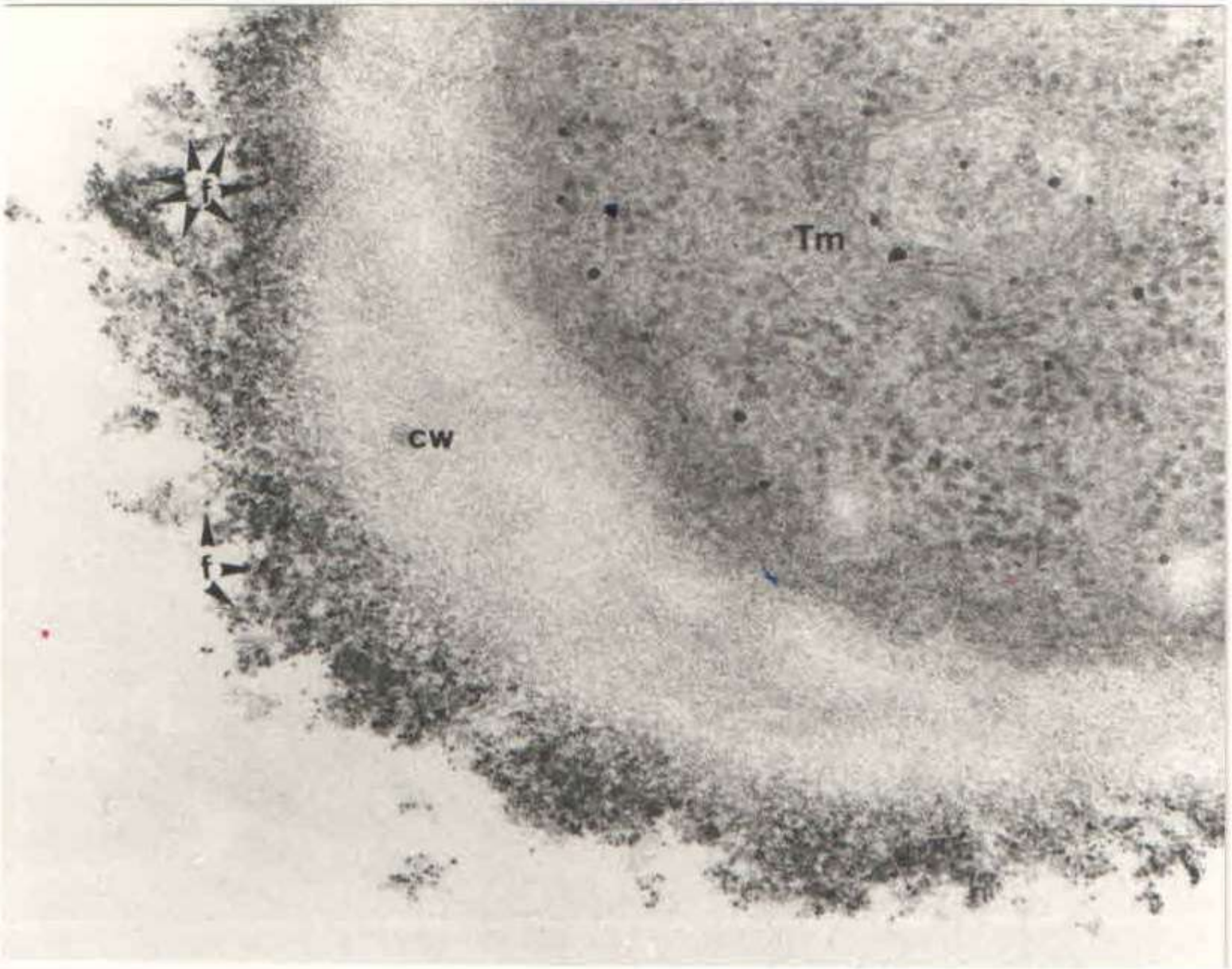


PLATE 81 : T. mentagrophytes, Untreated Control, I. Unabsorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F,Strong Ig-binding, Cell is healthy and normal (O.M. 30000 x, F.M. 87000 x, 23371).

Some Suggestions (1)

- ***Research should be conducted under constant supervision***
- ***Refresher courses should be conducted for supervisors & researchers***
- ***A course on 'Research techniques' should be compulsory for master students***
- ***Change the mindset of the students/researchers not to duplicate research conducted in developed countries. Highlight importance of relevant research***
- ***Motivate students to be a researchers (analytical minded) even though the aim of the student is not to go for research-oriented jobs***

Suggestions (2)

- ***Supervisors should take great pain in explaining all details of the changed/new methodologies. It is important to NOT assume anything; even though things may appear to be totally obvious***
- ***There should be a good communication with respect to providing full details of the experimental protocols and objectives of the project between the researcher and the Centralized Laboratory analysing samples***
- ***Extensive literature search before starting to design an experiment. The researcher should have clear ideas on limitations of methods, techniques and tools before conducting experiment***
- ***Before starting to design an experiment, important to make the hypothesis or question very clear. Avoid confounding by answering one question after the other. Analyse and interpret results according to the initial hypothesis***

Suggestions (3)

- ***The researcher should have an understanding of the technical background of the equipment***
- ***Before starting on in vitro or in vivo experiment, make sure that enough consumable material is available***
- ***Students should not be spoon fed nor left in isolation. They should develop protocols and organise research which should be discussed in a group. Result discussion and data presentation skills should be improved.***
- ***Proper selection of professors who teach and guide students at the post graduate level ('mediocrity breeds mediocrity').***
- ***Researcher should ensure use of proper statistical design and validated methods. Free communication with supervisor and group members should be encouraged***

Recap

- ***Improper statistical designs***
- ***Poor knowledge on the principles of equipment use***
- ***Poor knowledge on the principles of bioprocesses***
- ***Poor knowledge on the use of standards and other quality control principles***
- ***Improper general laboratory practices***
- ***Lack of information on laboratory safety issues***
- ***Improper presentation of results***
- ***Poor presentation skills***

The Challenge

- ***In addition to enhancing theoretical and practical knowledge on biological systems and research methodology, build confidence enabling better use of common sense***