IMMUNOLOGICAL TECHNIQUES IN CLINICAL DIAGNOSIS

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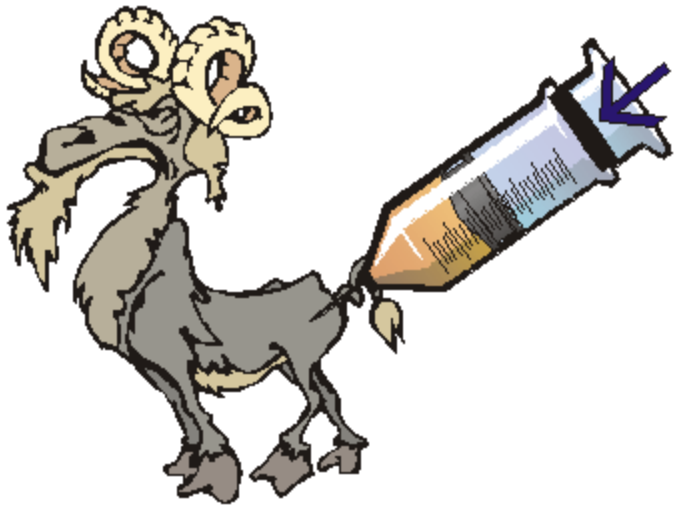
ANTIGEN PRODUCTION

- *Method of choice depends upon organism, location of antigens in cell and type of antigen*
- *Cell fractionation*
- *Separation of desired antigen*
- *Purification*
- *Storage in small amounts*
- *Immunization of test animal*

ANTIBODY PRODUCTION



Desired quantity of antigen is injected into test animal at multiple points with Freund's complete Adjuvant

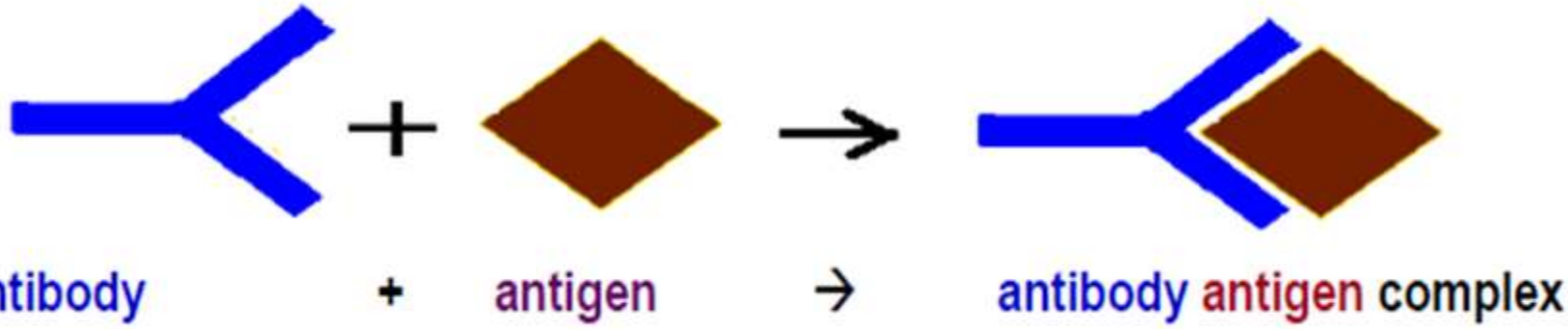
Booster doses are given after testing antibody production on each dose of immunization

Last doze is given with Freund's Incomplete Adjuvant

Animal is bled aseptically by heart puncture

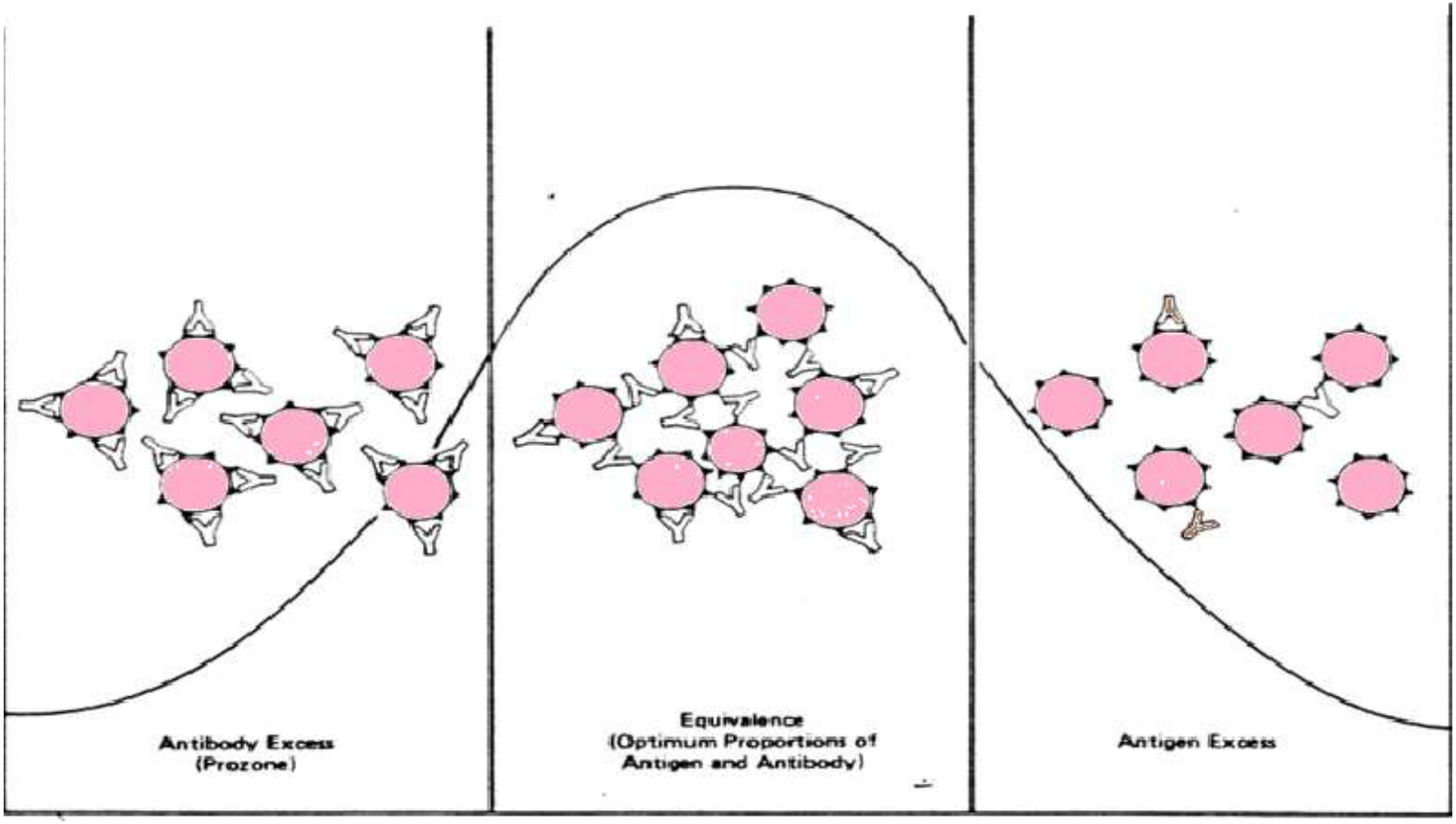
Antibodies are separated by centrifugation and stored in small quantities at -20^{o}C

ANTIGEN-ANTIBODY REACTION



Antigen-Antibody-Ratio

determines visible insoluble precipitate

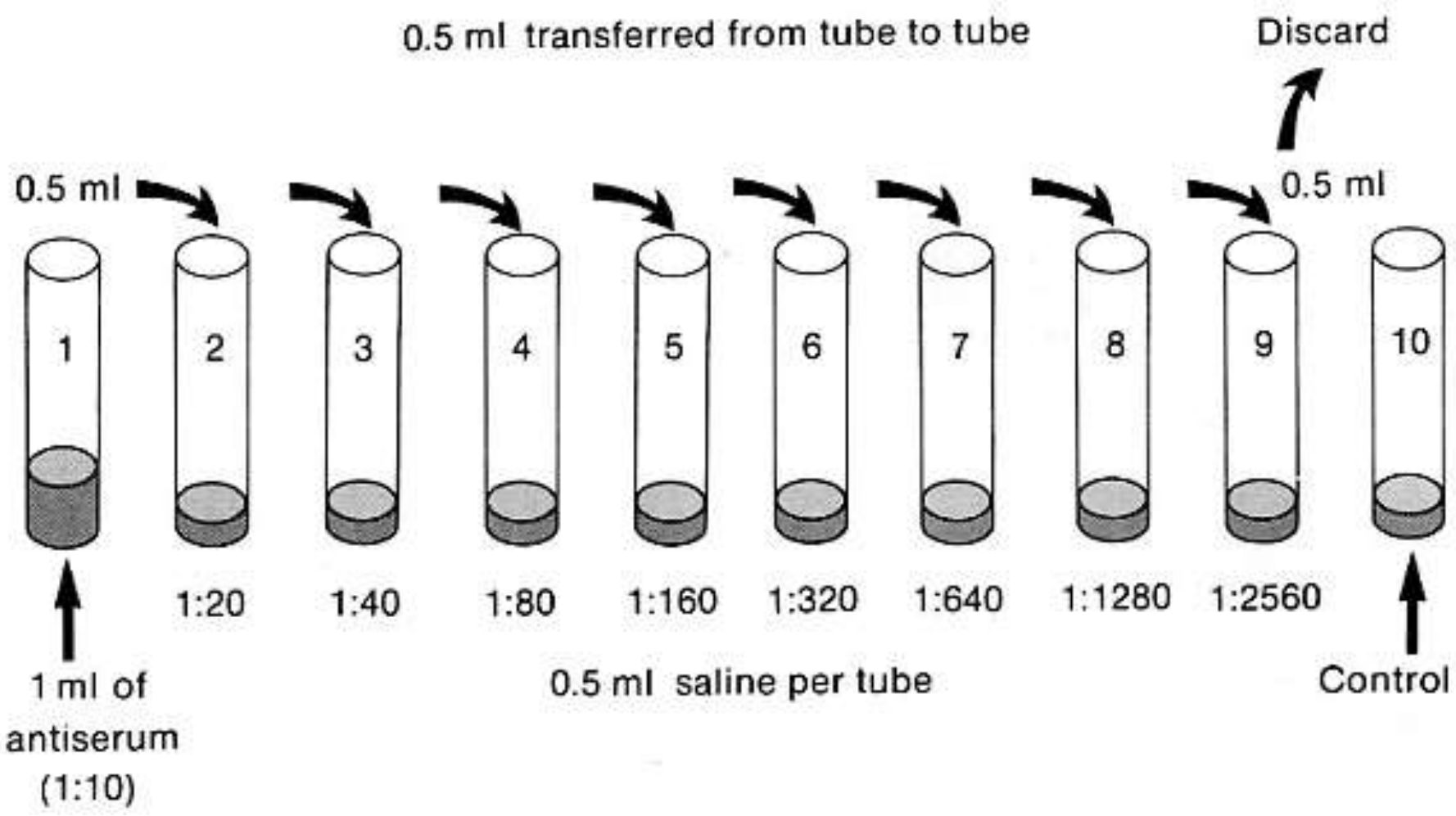


Prozone : Ab excess

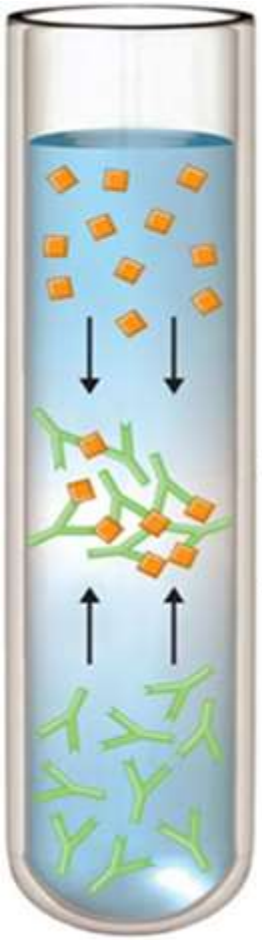
Zone of equivalence – showing insoluble precipitate

Post-zone – excess of Ag

Basic Principle of Dilution



Precipitation Reaction

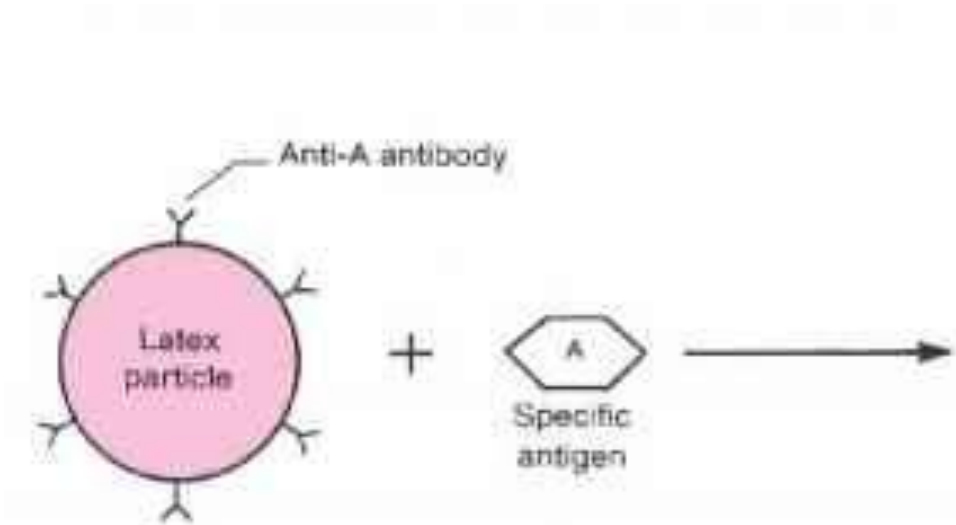


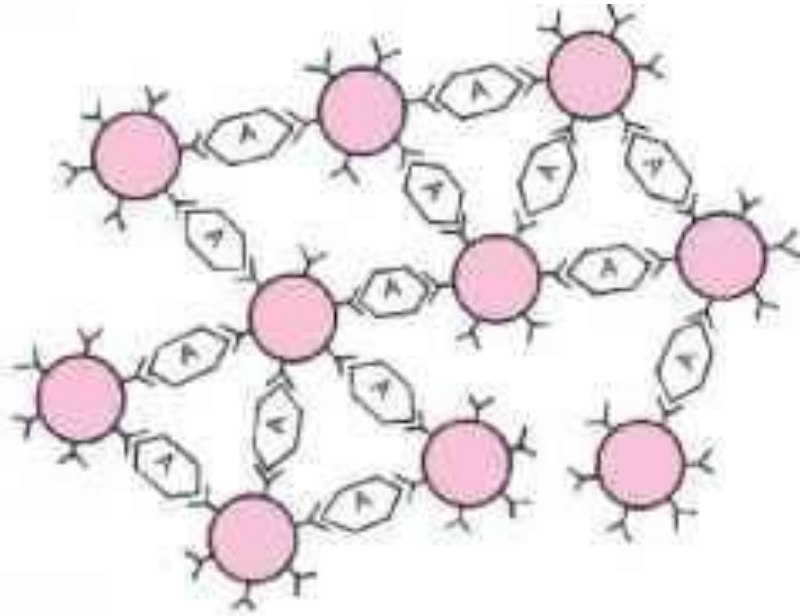
Antigens
(soluble)

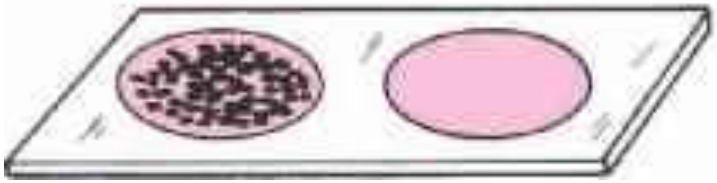
Zone of equivalence:
visible precipitate

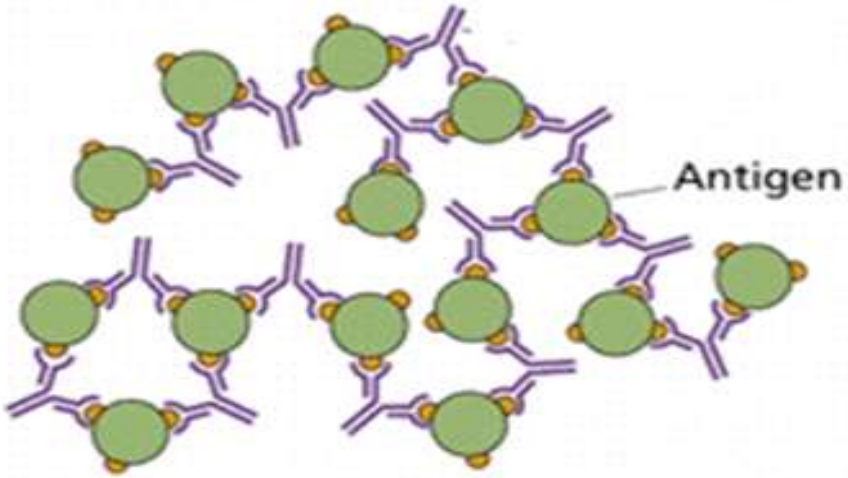
Antibodies

Agglutination Reaction

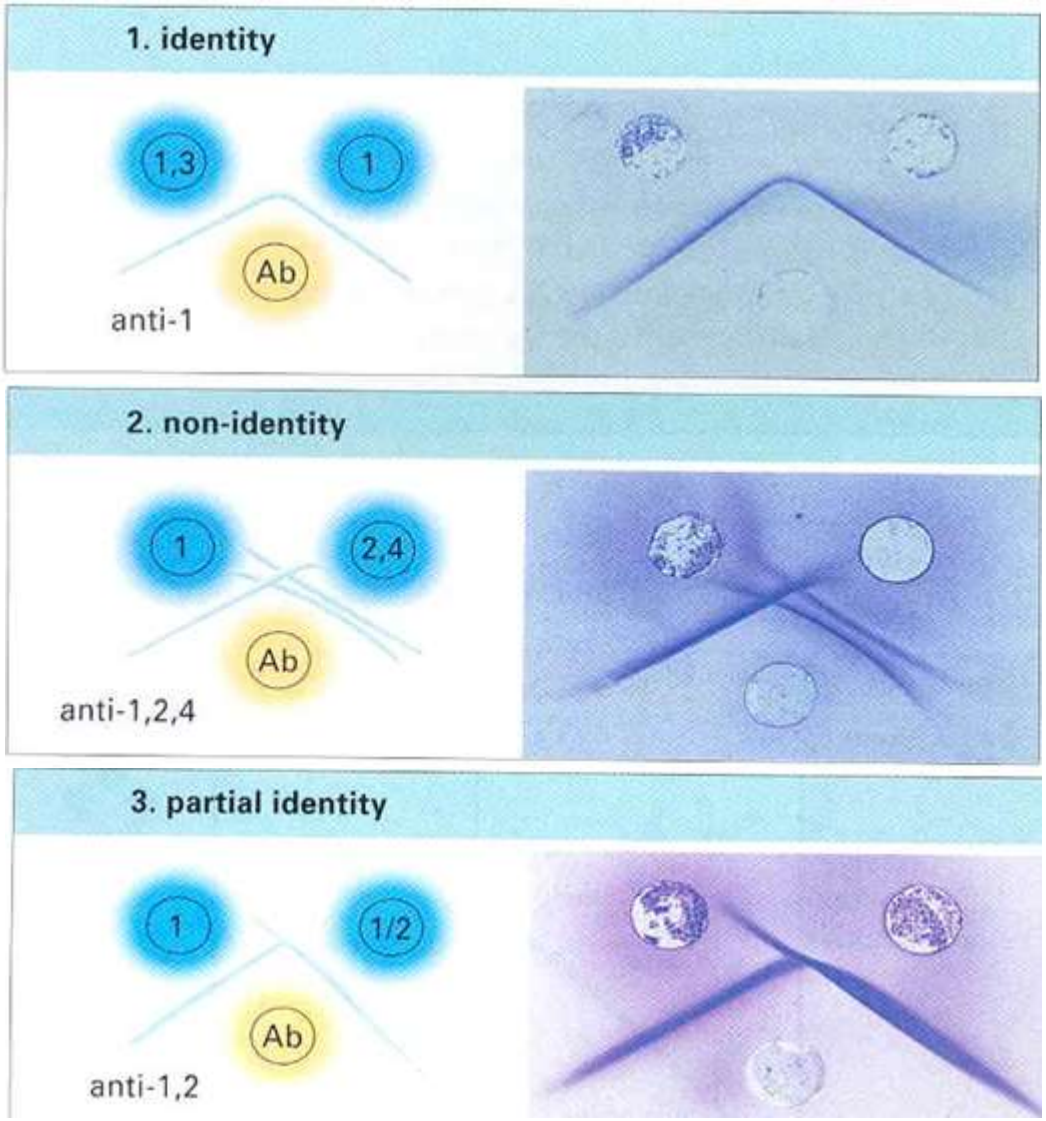


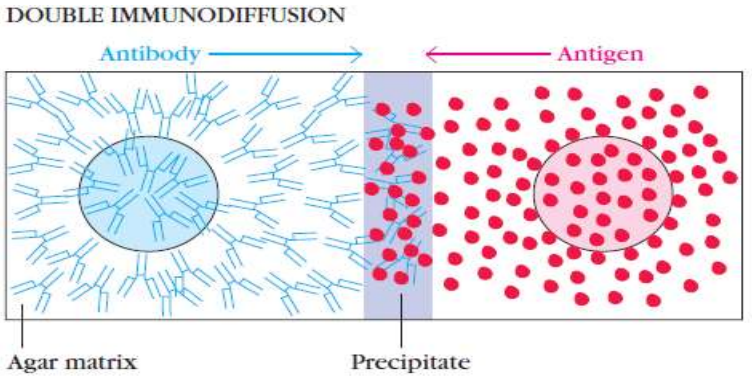


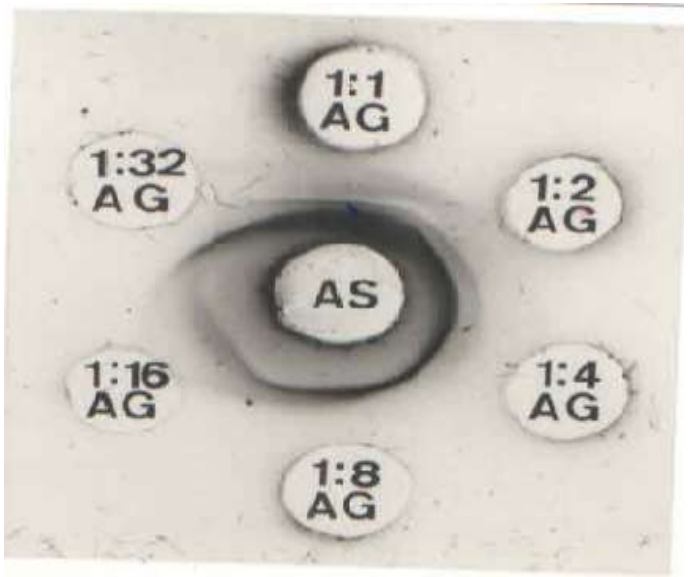




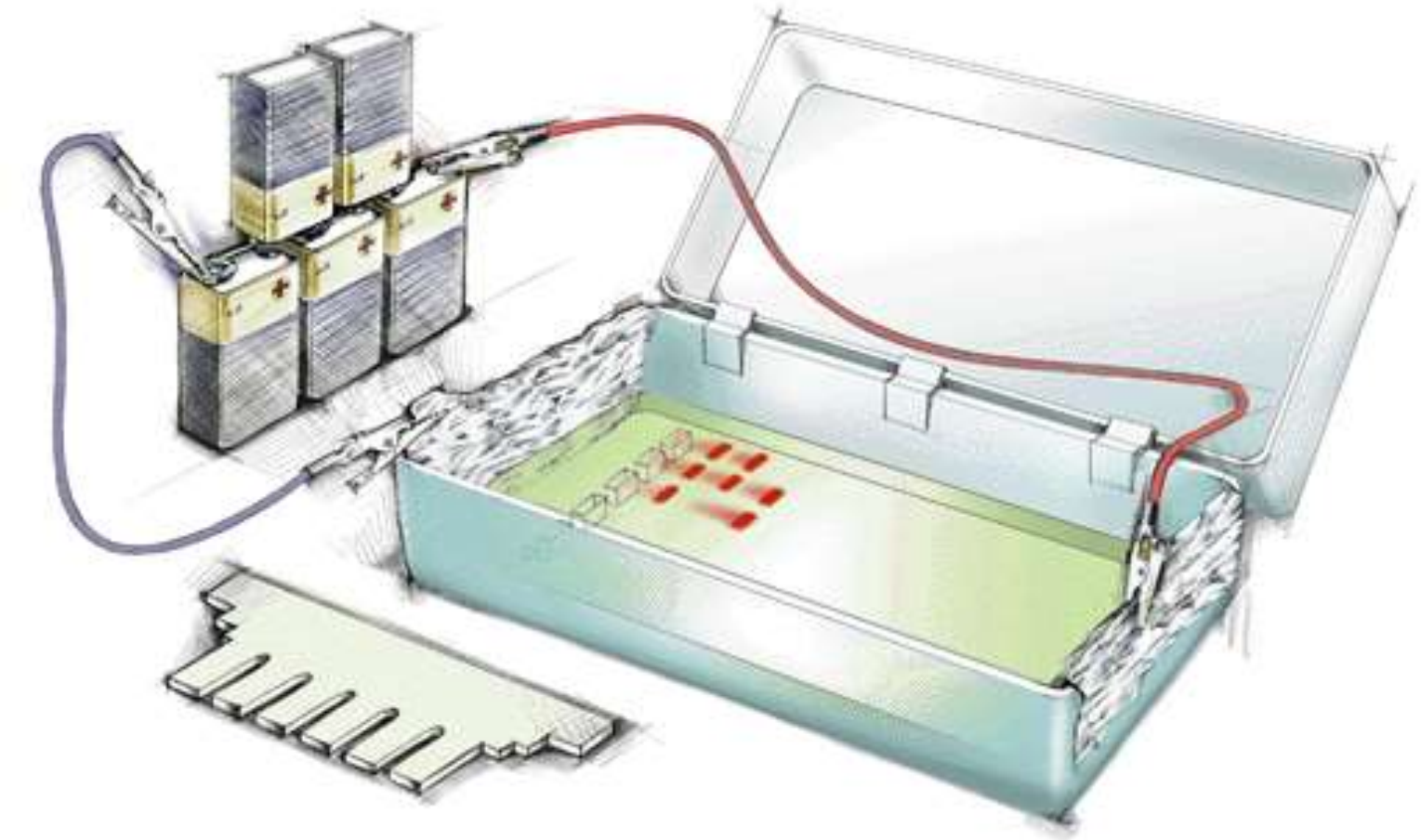
Ochterlony Immuno-double Diffusion



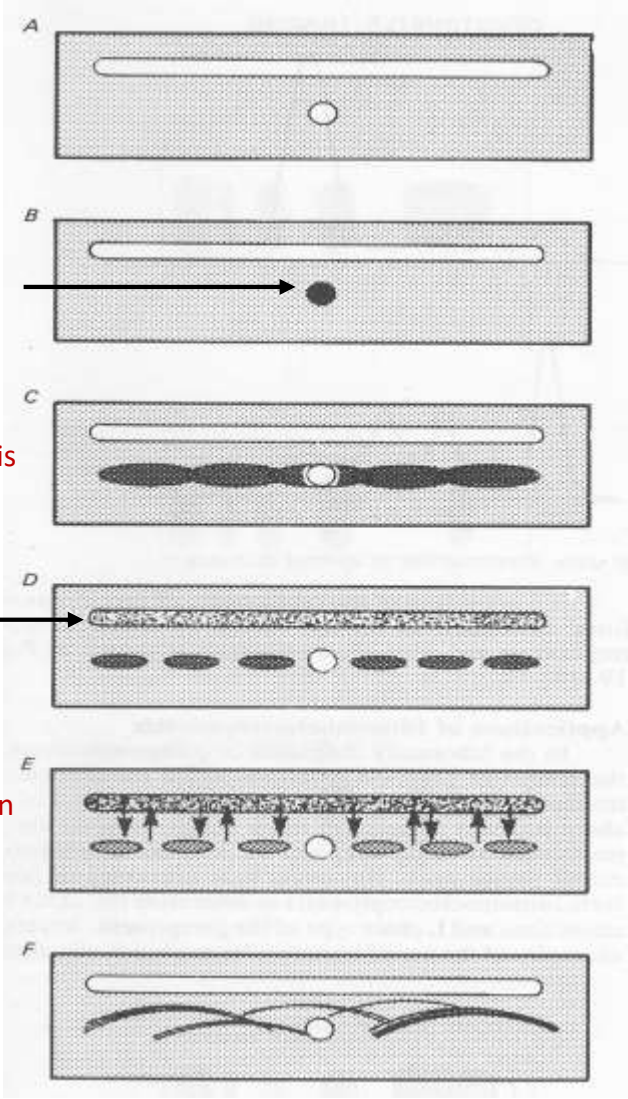




Principle of Electrophoresis

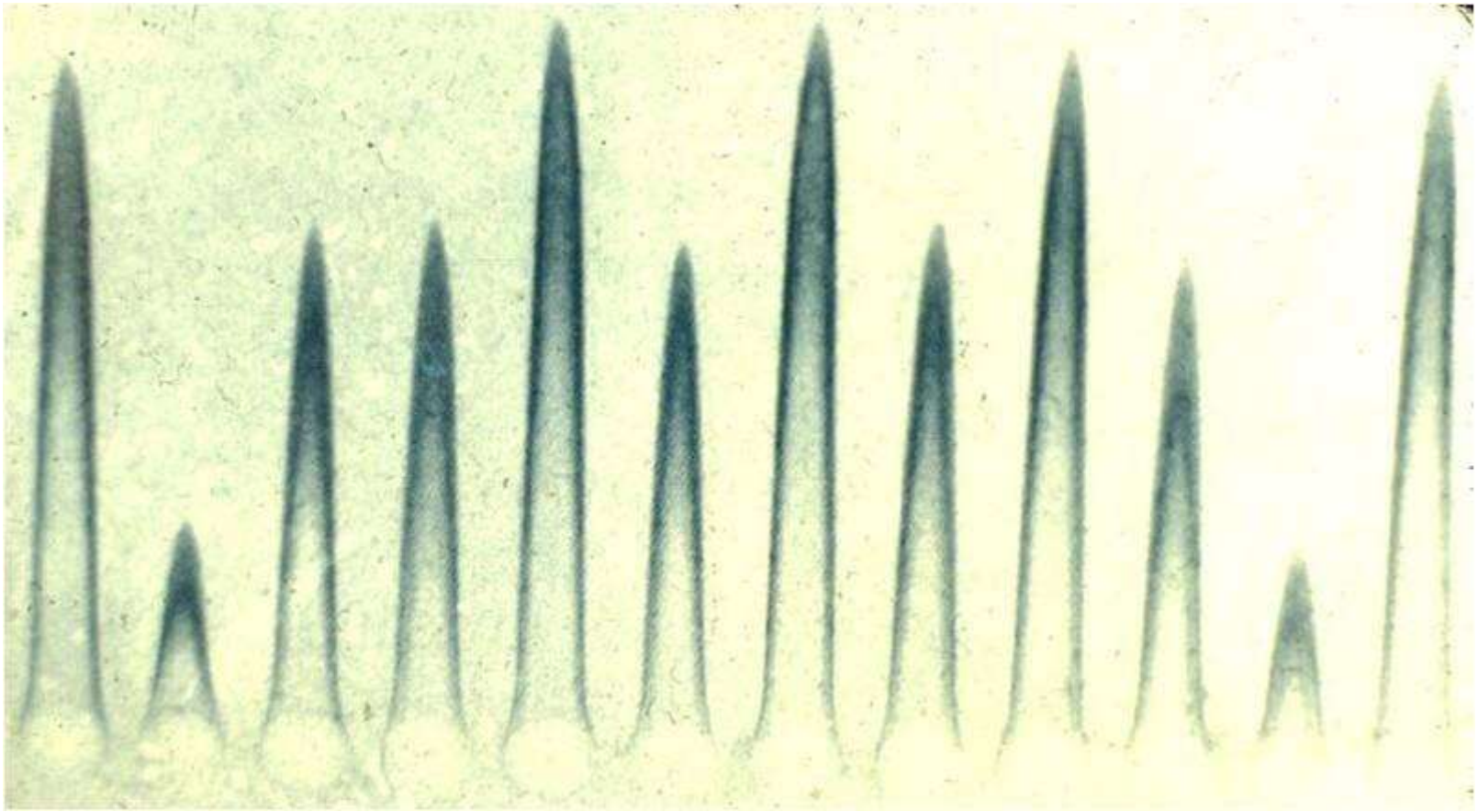


Immunoelectrophoresis



It combines electrophoresis separation, diffusion and precipitation of protein antigens

Rocket Immunoelectrophoresis

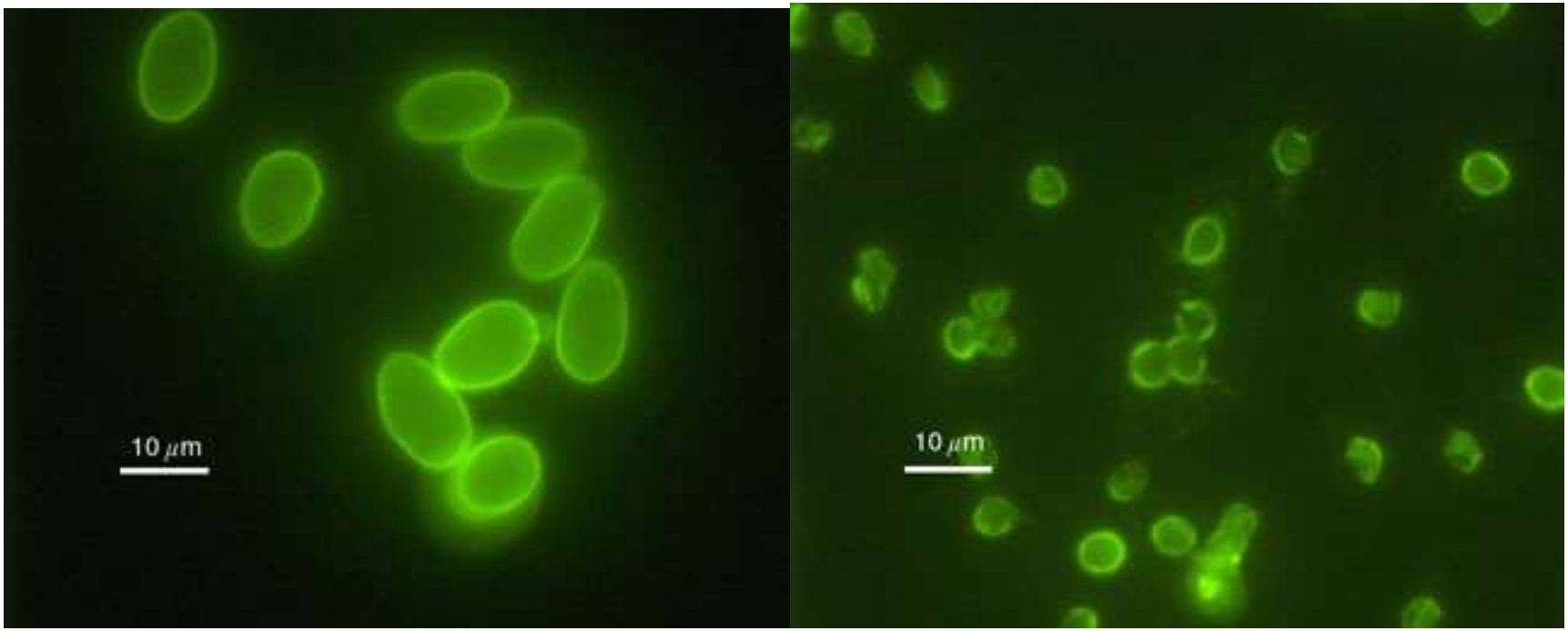


Crossed Immunoelectrophoresis

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

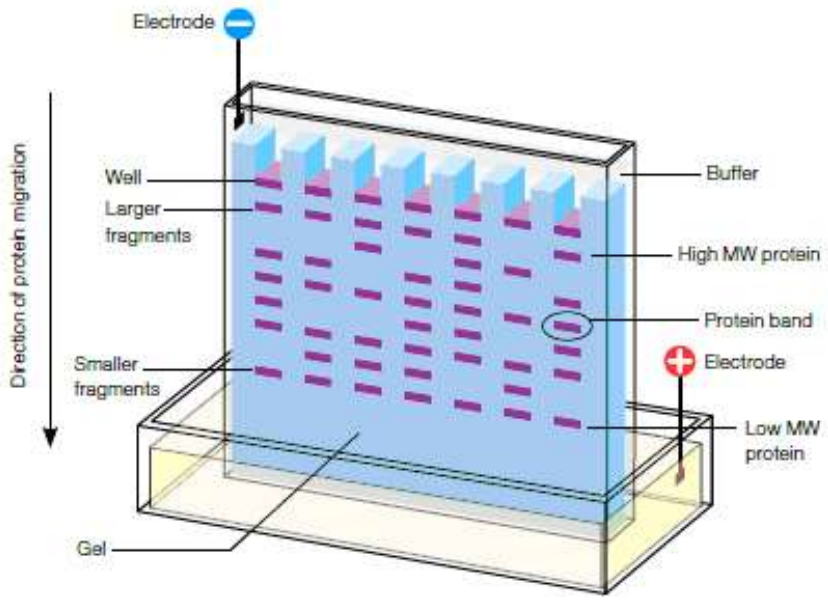


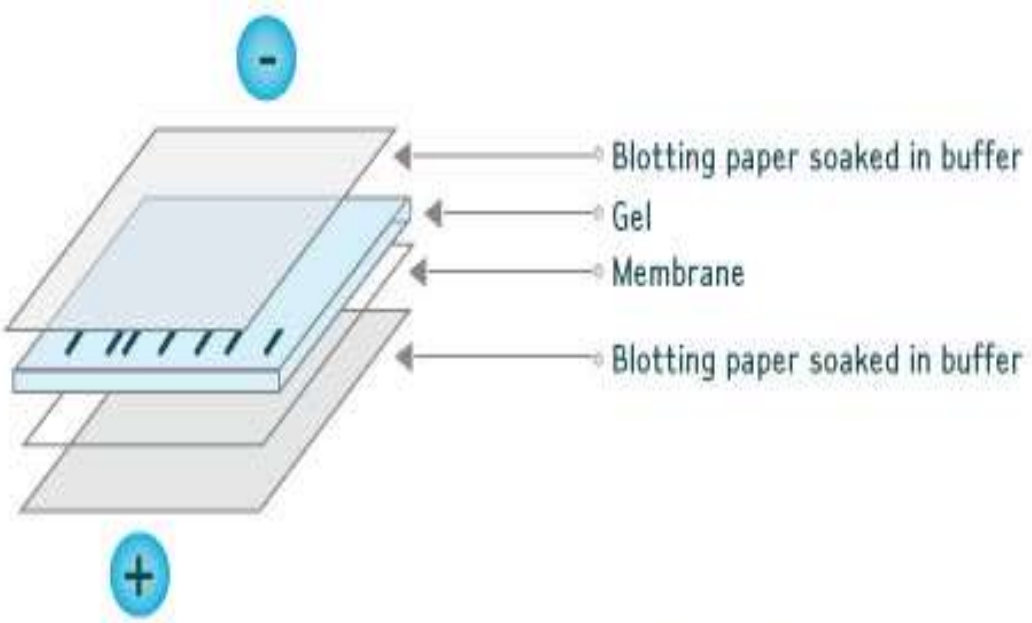
Fluorescent Antibody Staining
***Giardia* (left)**
***Cryptosporidium* (right)**

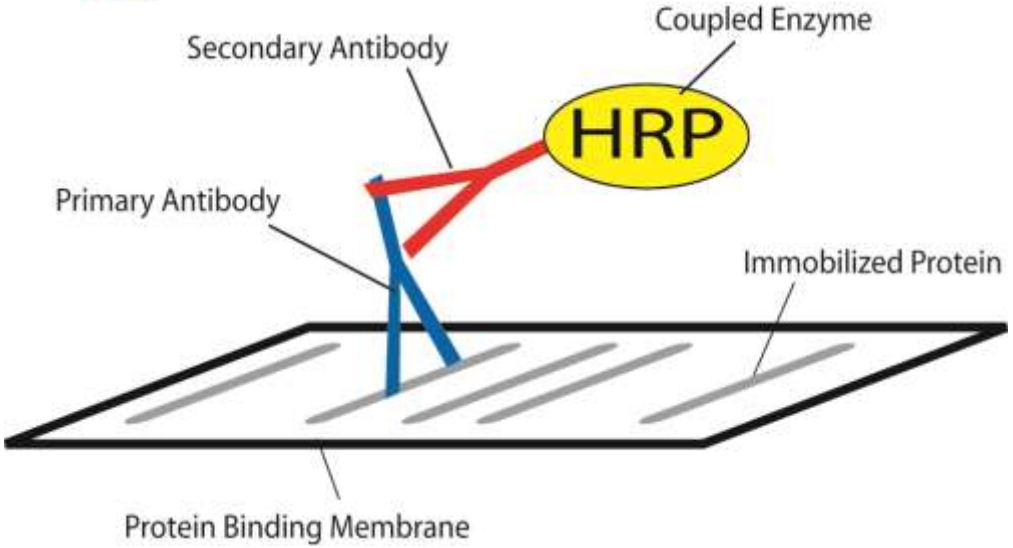
Immunoblotting

- ***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***

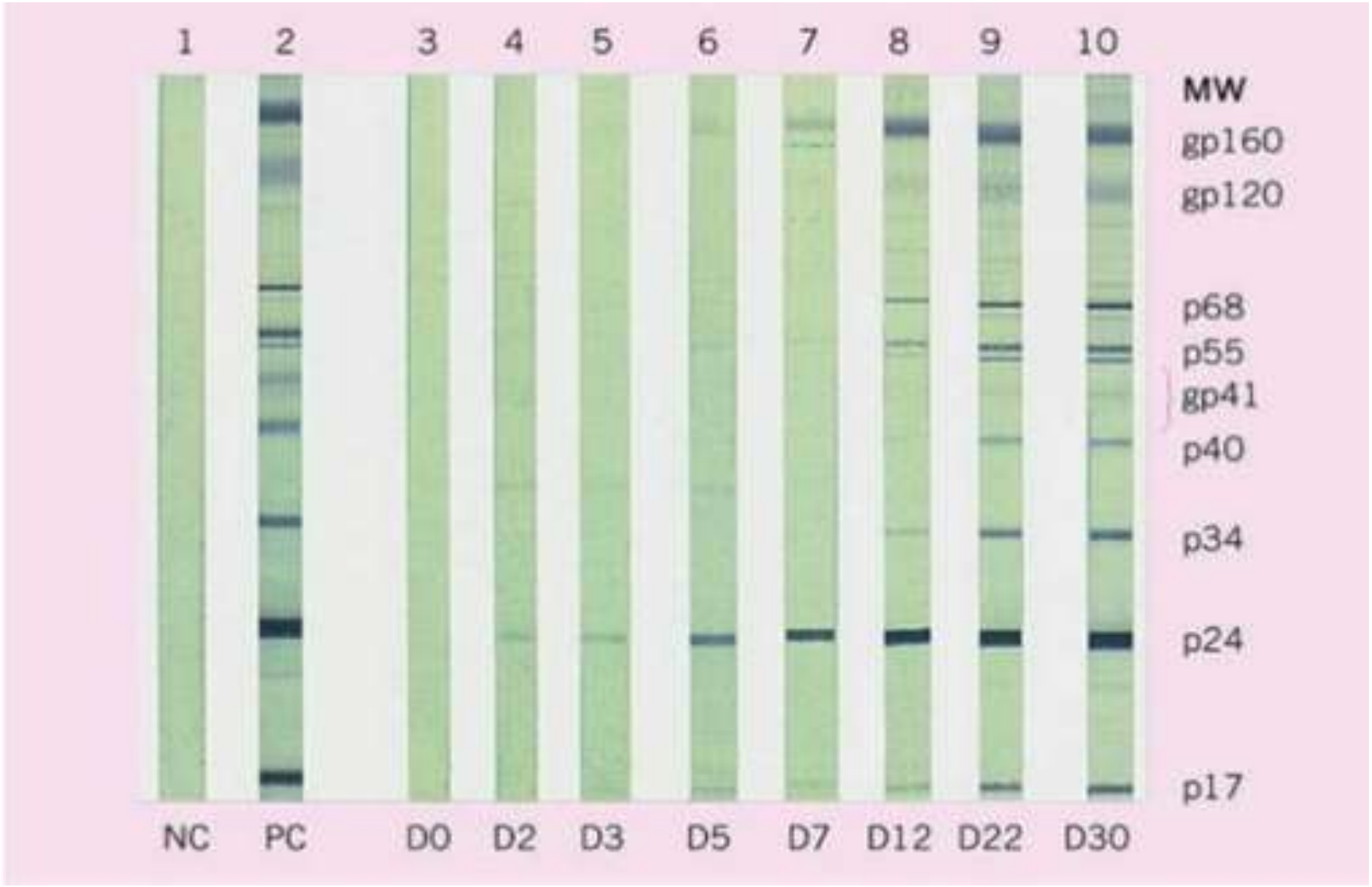
Major Steps in Immunoblotting







Typical Western Blot of HIV patients



Immunoblot-when proteins are not dominant antigens

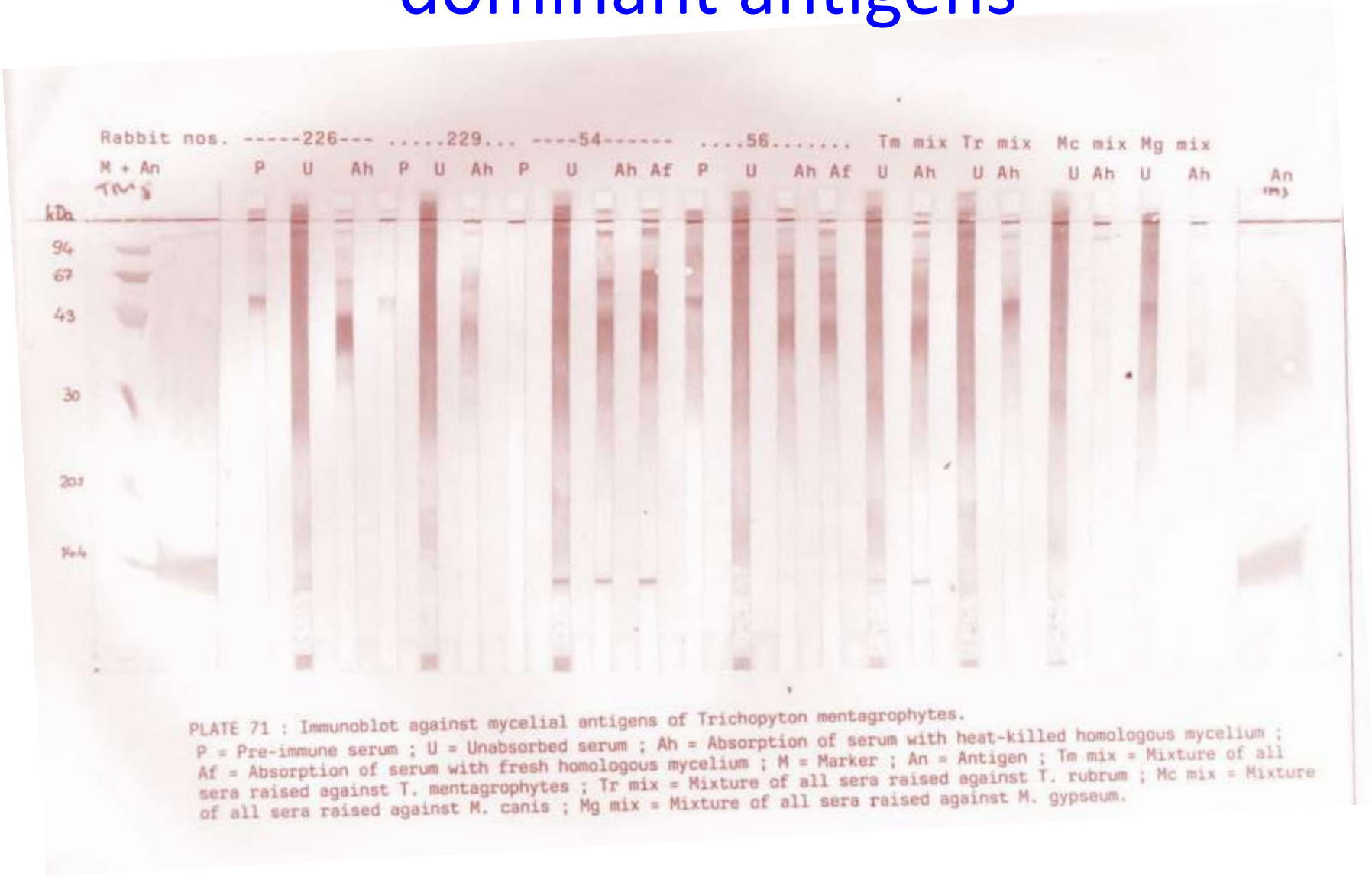
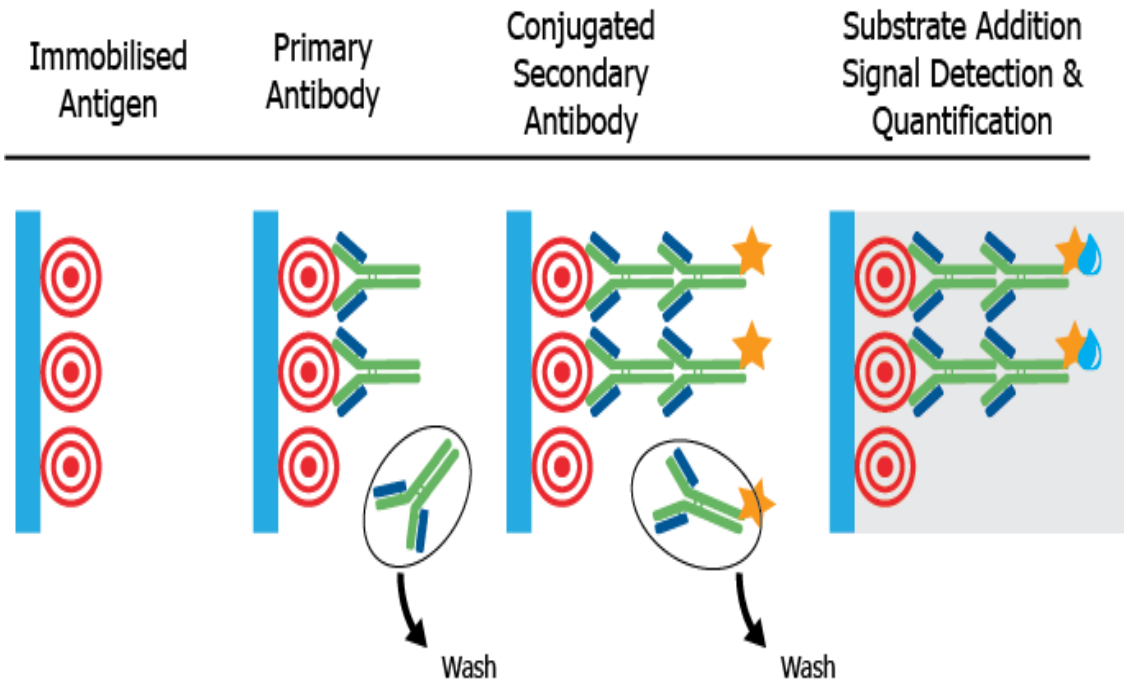


PLATE 71 : Immunoblot against mycelial antigens of Trichopyton mentagrophytes. P = Pre-immune serum ; U = Unabsorbed serum ; Ah = Absorption of serum with heat-killed homologous mycelium ; Af = Absorption of serum with fresh homologous mycelium ; M = Marker ; An = Antigen ; Tm mix = Mixture of all sera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

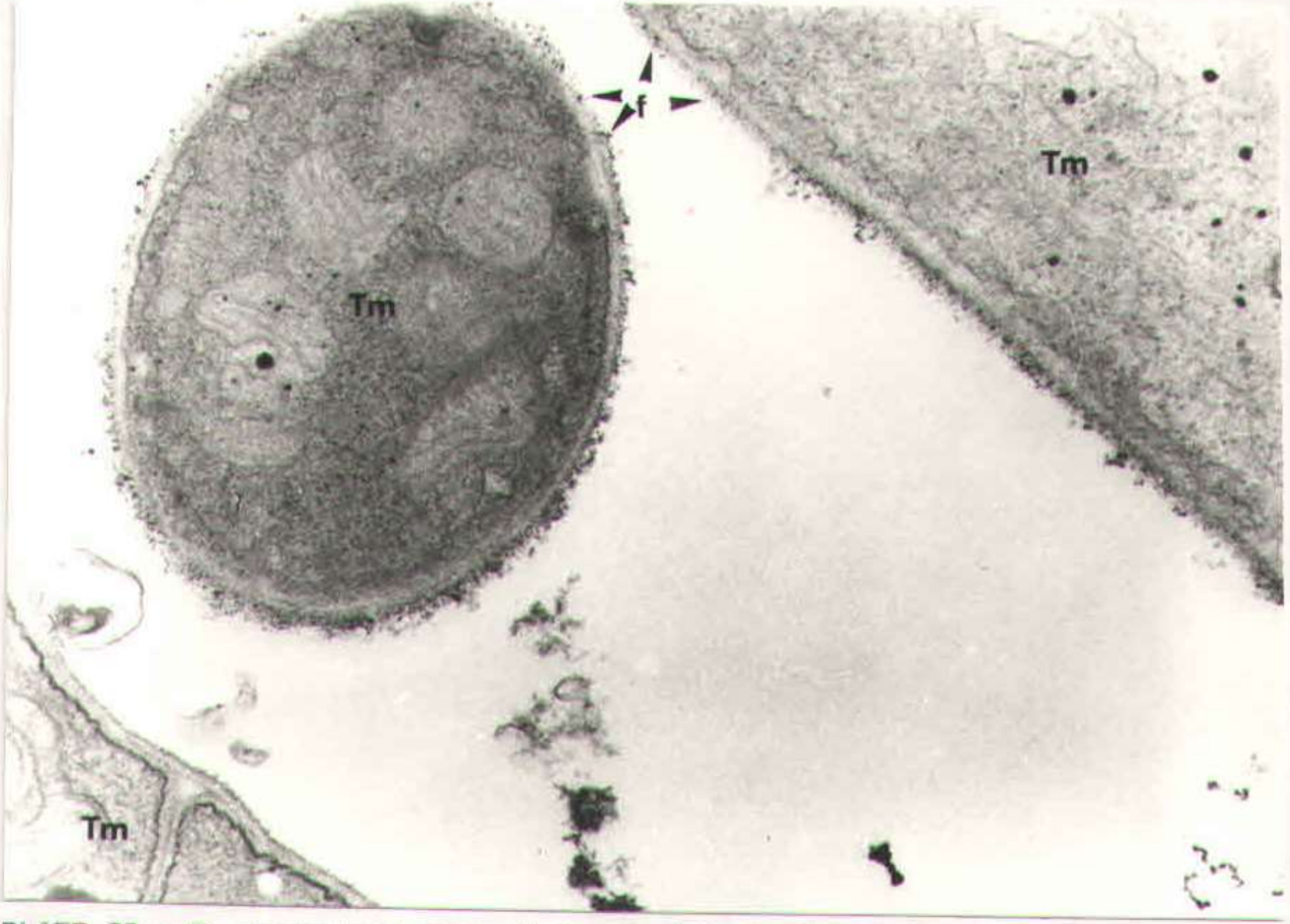


PLATE 83 : T. mentagrophytes, Untreated Control, I. Absorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F ,´Moderate Ig-binding , Cells are healthy (O.M. 10000 x, F.M. 26500 x, 23696).

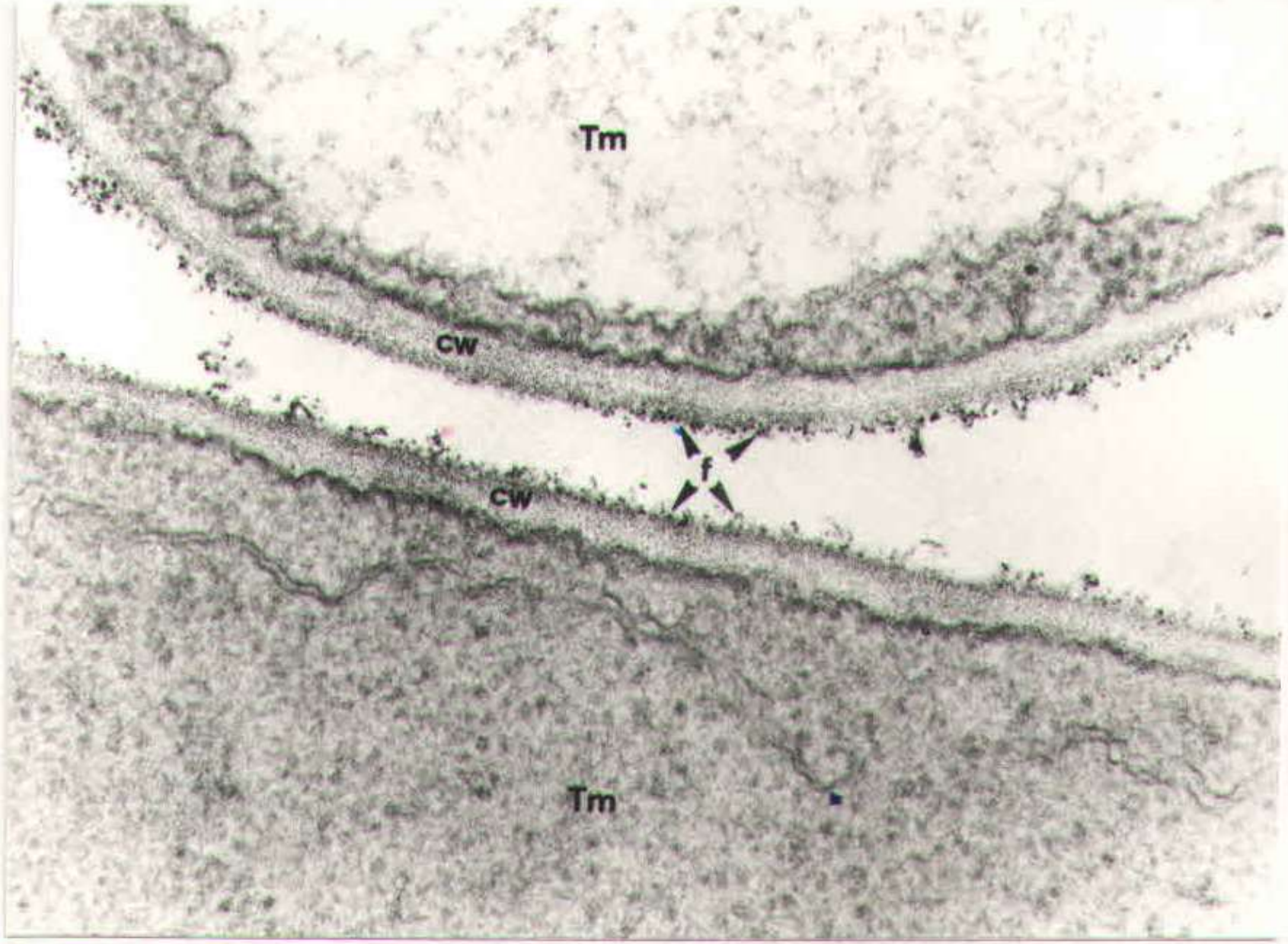


PLATE 84 : T. mentagrophytes, Untreated Control, I. Absorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F , Moderate Ig-binding , Cells are healthy (O.M. 30000 x, F.M. 80800 x, 23692).

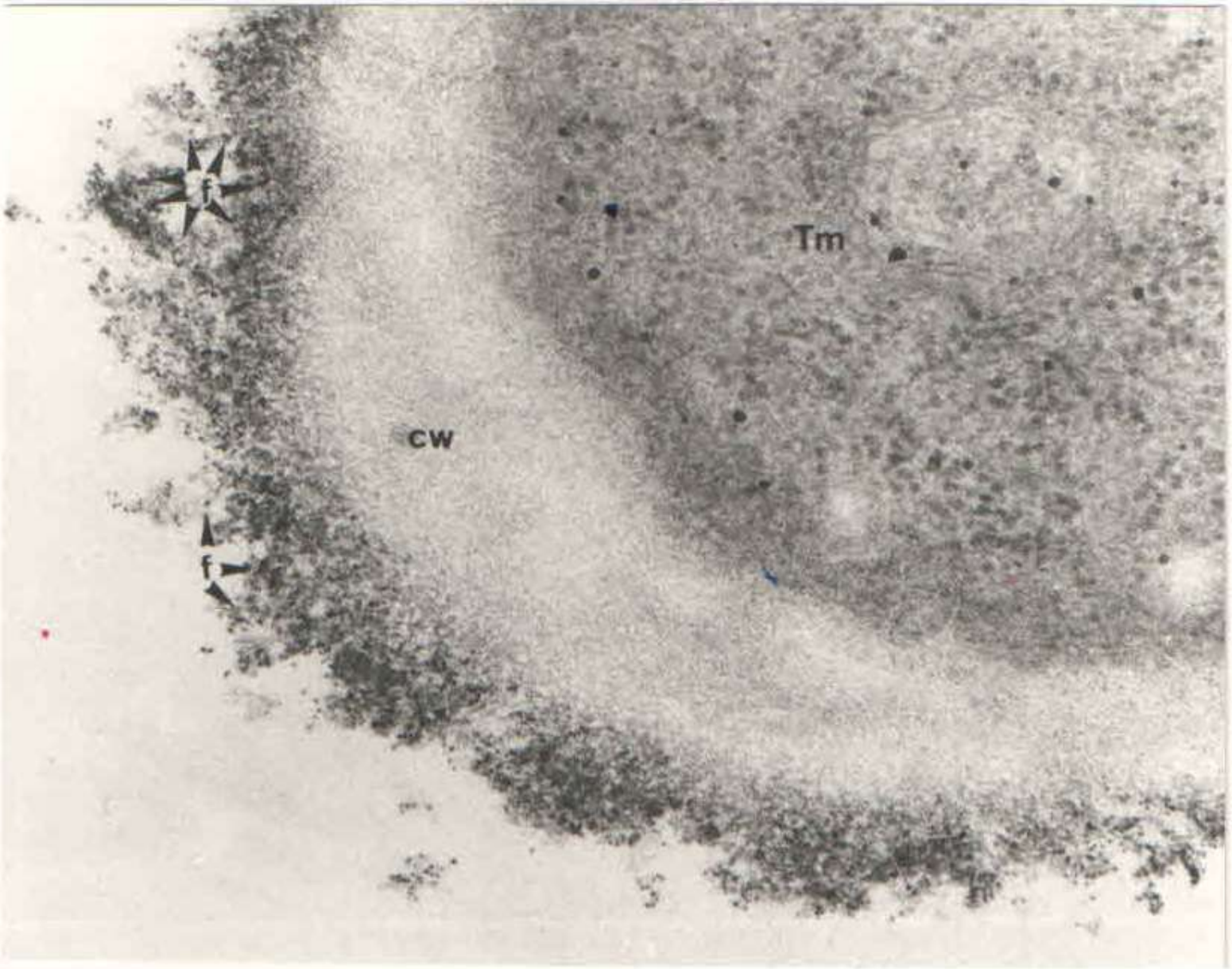


PLATE 81 : T. mentagrophytes, Untreated Control, I. Unabsorbed, Rabbit-Anti-T.m. II. Anti-Rabbit-Ig-F,Strong Ig-binding, Cell is healthy and normal (O.M. 30000 x, F.M. 87000 x, 23371).

THANKING YOU