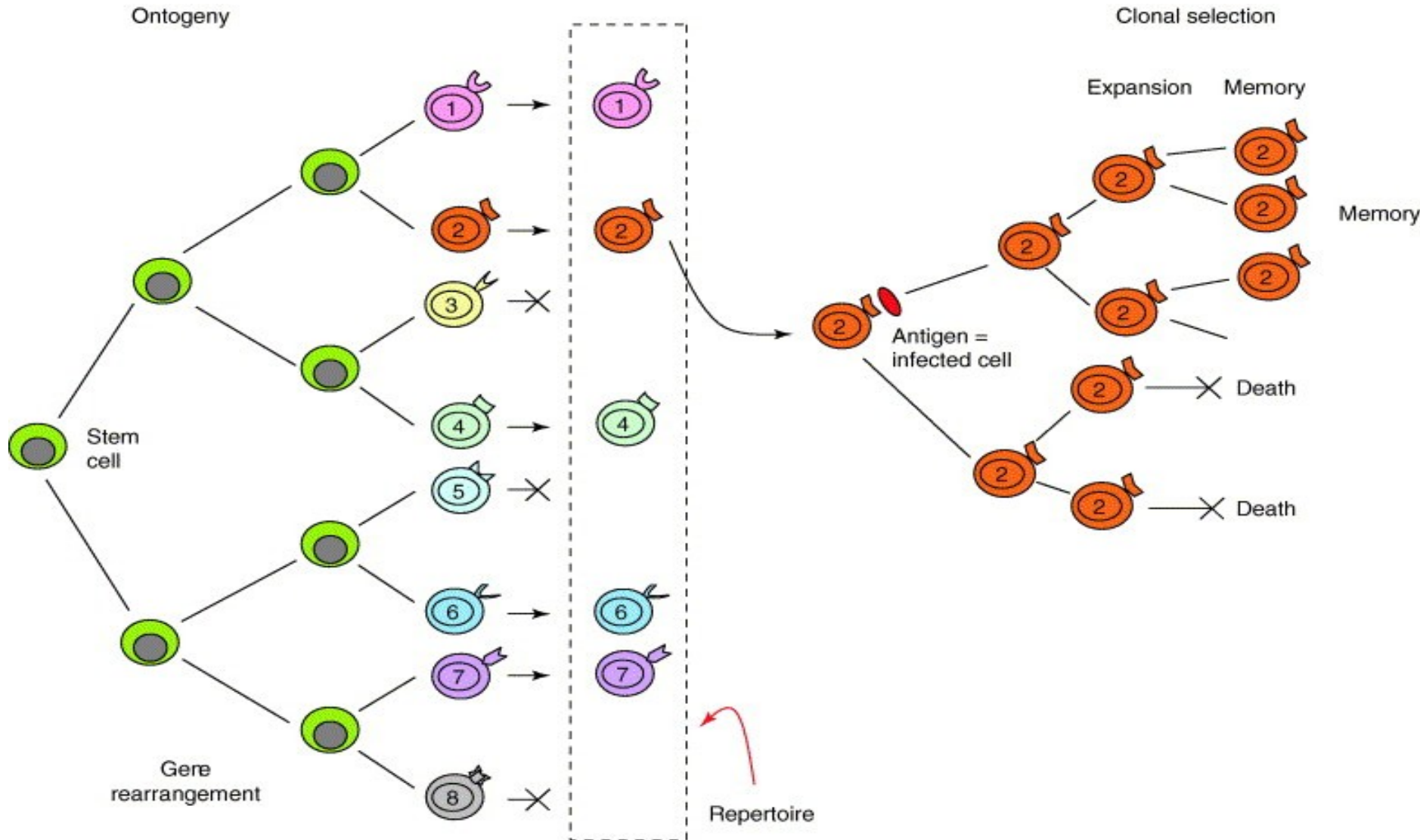


Serological reactions

(Polyclonal) antisera

- Obtained a from animals (rabbits, goats, horses) after repeated immunisation by antigen.
- Markedly polyreactive – antibodies bind to many epitopes of the antigen but also with other antigens.
- This is advantageous in „classical“ serological reactions (agglutination, precipitation).

Clonal selection theory



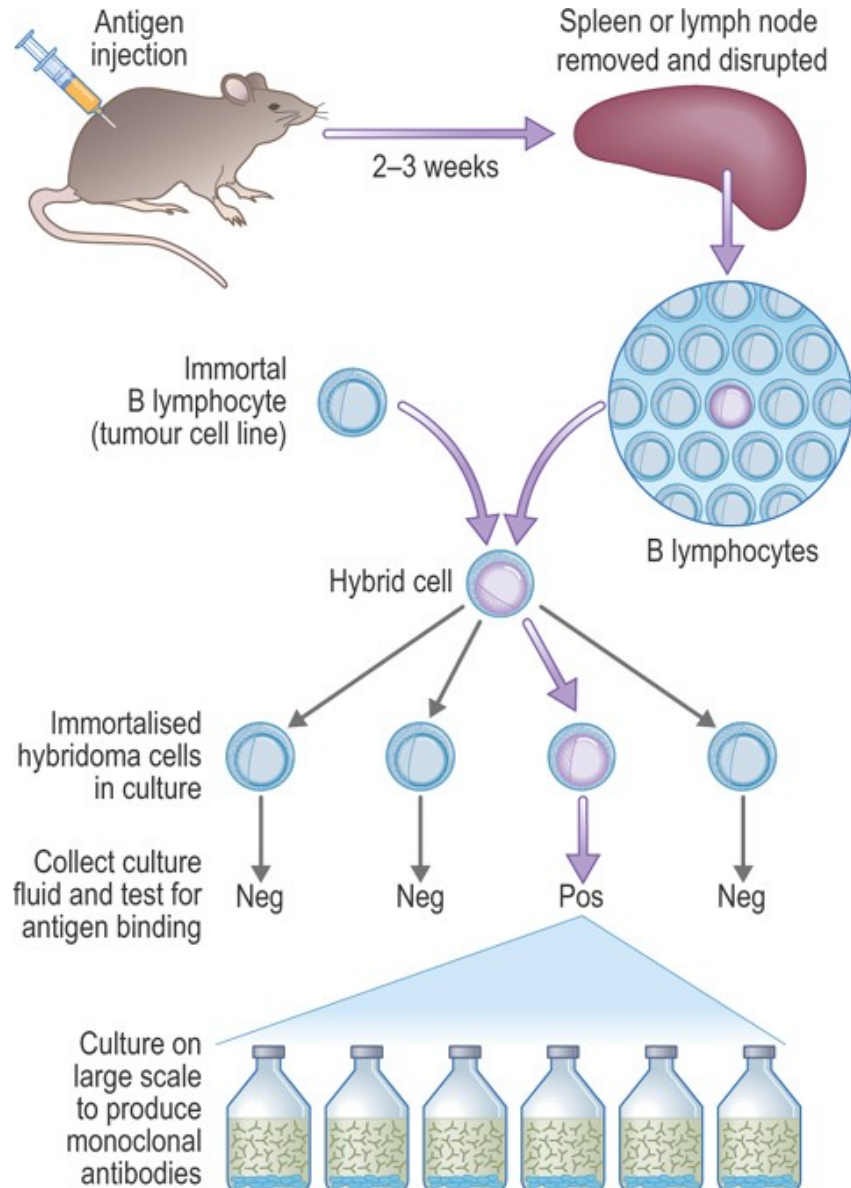
Myeloma

- Tumor derived from plasma cell
- The tumor cells retain the capacity to secrete immunoglobulins
- The secreted immunoglobulin is a **paraprotein** - all secreted molecules have the same variable region (= react with only one concrete epitope)

Monoclonal antibodies

- Prepared by immortalization of B-cells from immunized mouse.
- Hybridoma is composed of an antigen-specific B- cell and mouse myeloma cell.
- Produced antibodies are strictly monospecific and therefore cannot be used in several „classical“ serological reactions (agglutination, precipitation).

Preparation of monoclonal antibodies



Laboratory use of monoclonal antibodies

- Highly specific agent used for ELISAs, RIAs, determination of cells surface antigens...
- Because they react only with a single epitope, number of „bridges“ is too low to overcome repulsive forces in classical reactions like agglutination or precipitation.

Clinical use of monoclonal antibodies

- Immunosuppressive treatment (anti CD3, CD54, CD20)
- Antinflammatory treatment –
 - Cytokine neutralisation (anti- $\text{TNF}\alpha$)
 - Adhesion molecules blockade (anti-LFA-1....)
- Anti-tumor treatment (anti-CD20, anti EGF..)
- Anti allergic treatment – (anti-IgE)
- Anti aggregation treatment (anti- gpIIb-IIIa – blocks activation of thrombocytes)

Two phases of serological reaction

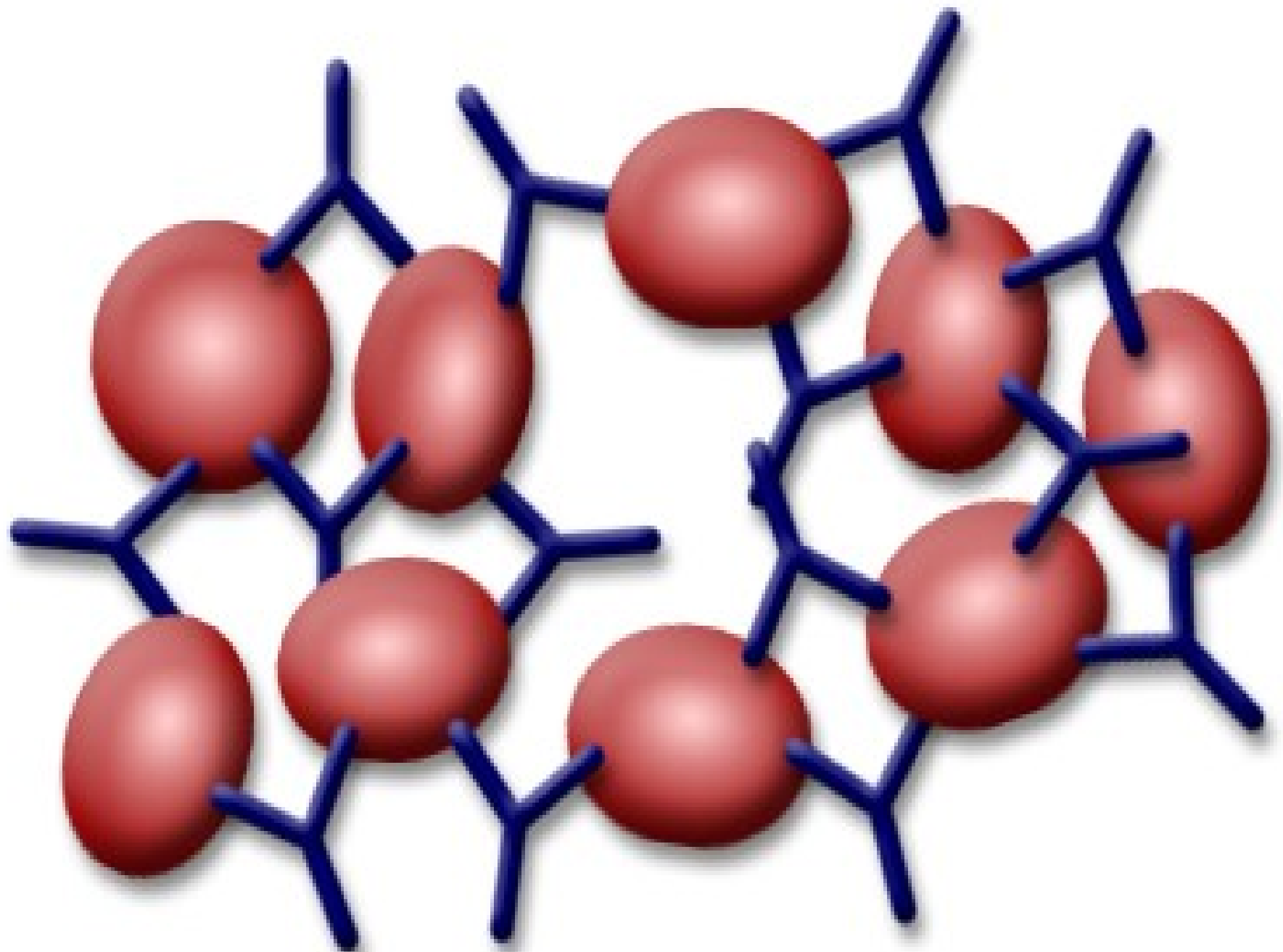
- Primary phase – concrete antibody (with its variable region must be present) binds to a concrete epitope.
= Specific phase of the reaction
- Secondary phase – visualization of the fact of previously occurred primary reaction.

Serological reactions

- Agglutination
- Precipitation
- Immunoassays
 - RIA
 - ELISA
 - Immunofluorescence
- Reactions based on activation of complement cascade by complex-antigen-antibody
- Reactions based on neutralisation of some biologic effect of antigen

Agglutination

- Reaction between antiserum and corpuscular antigen (erythrocyte, bacterium, latex corpuscle). The corpuscles are clumped together, which morphologically expressed as agglutinate.

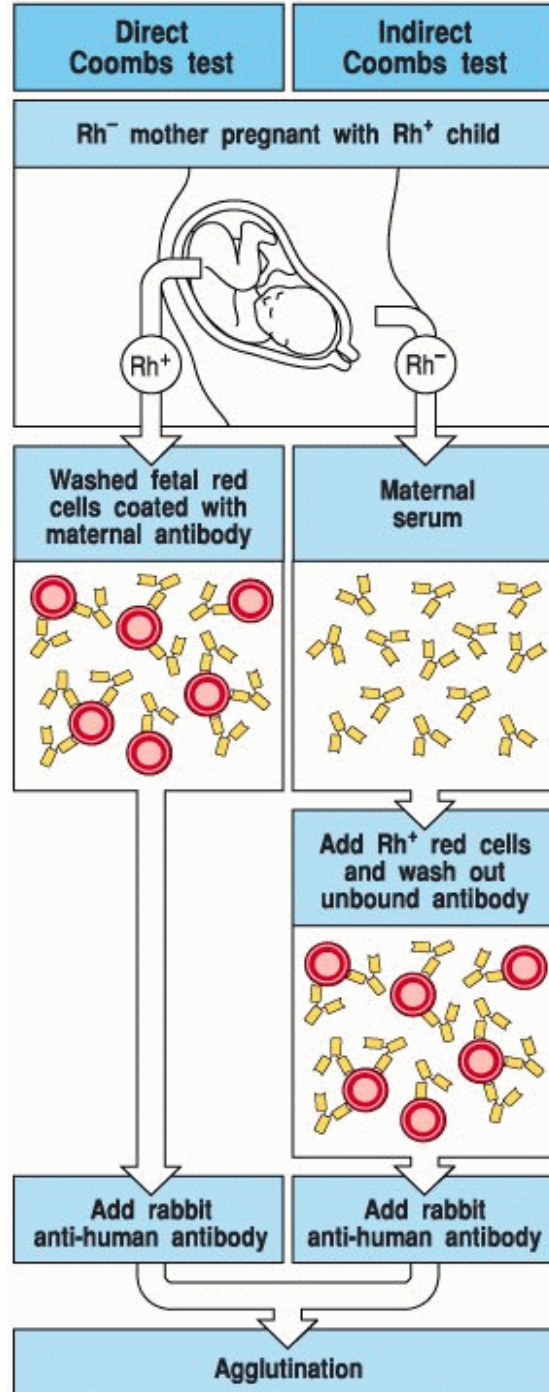




- **Complete antibodies:** after reaction with antigen cause visible agglutination or precipitation reaction
- **Incomplete antibodies:** despite the fact that the reaction between epitope and antibody occurred, the agglutinate or precipitate cannot be detected.

Cause: monovalent antibody (IgA), low number of bridges between antigens, to intense repulsive forces between antigens...

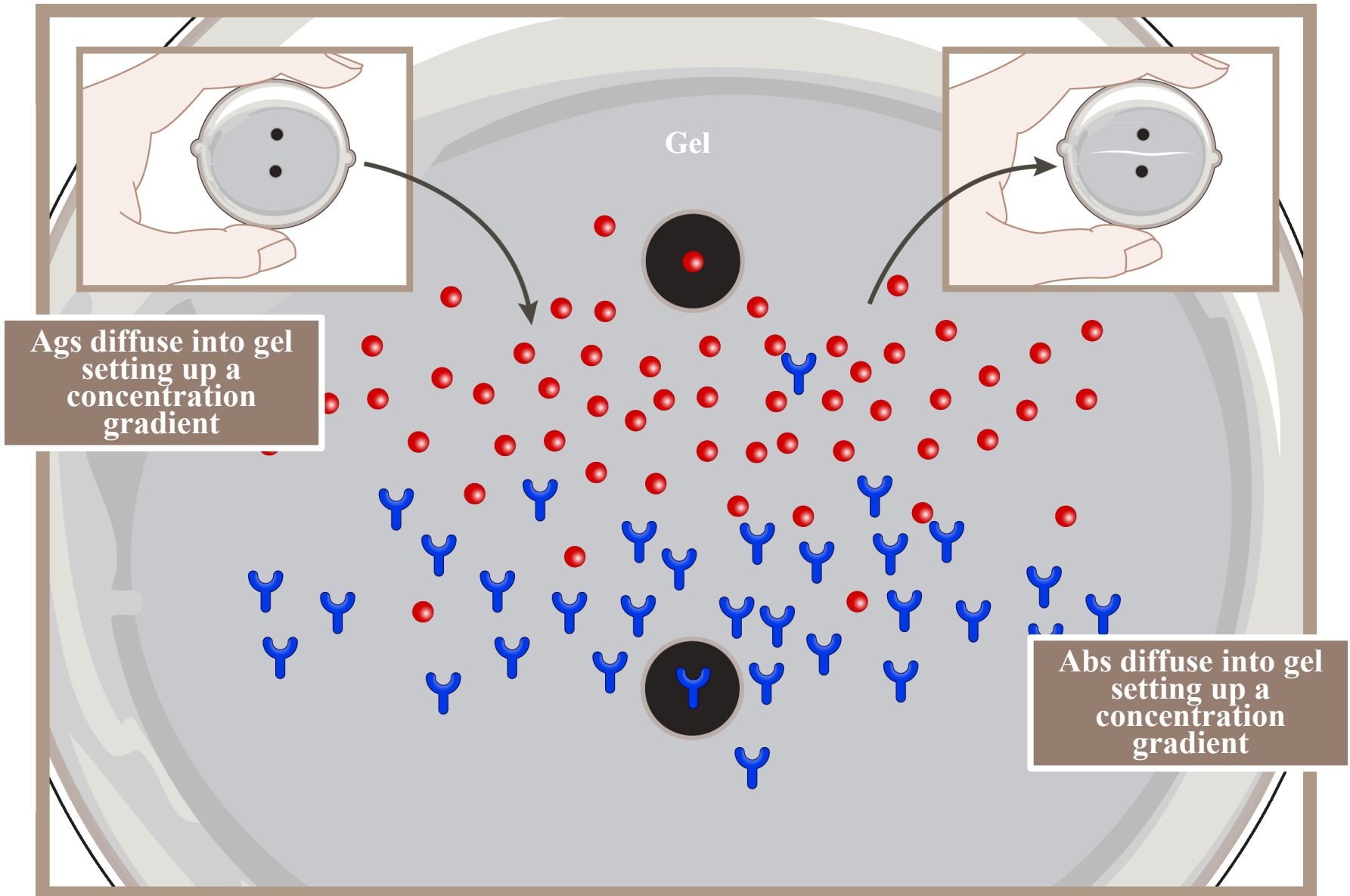
Coombs test



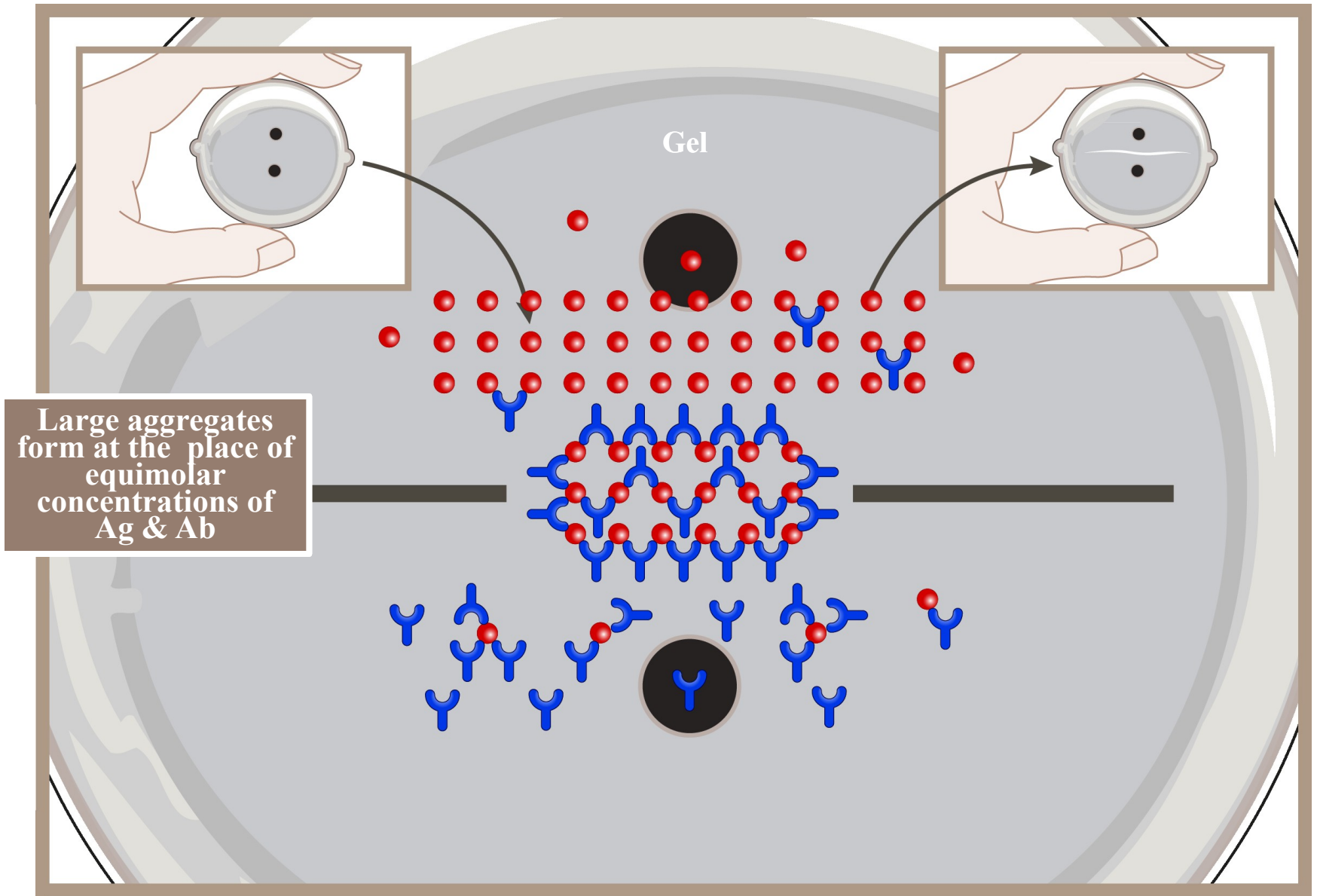
Precipitation

- Reaction between polyclonal antiserum and soluble (molecular) antigen. A complex lattice of interlocking aggregates is formed. If performed in a solution the precipitate falls out of the solution.

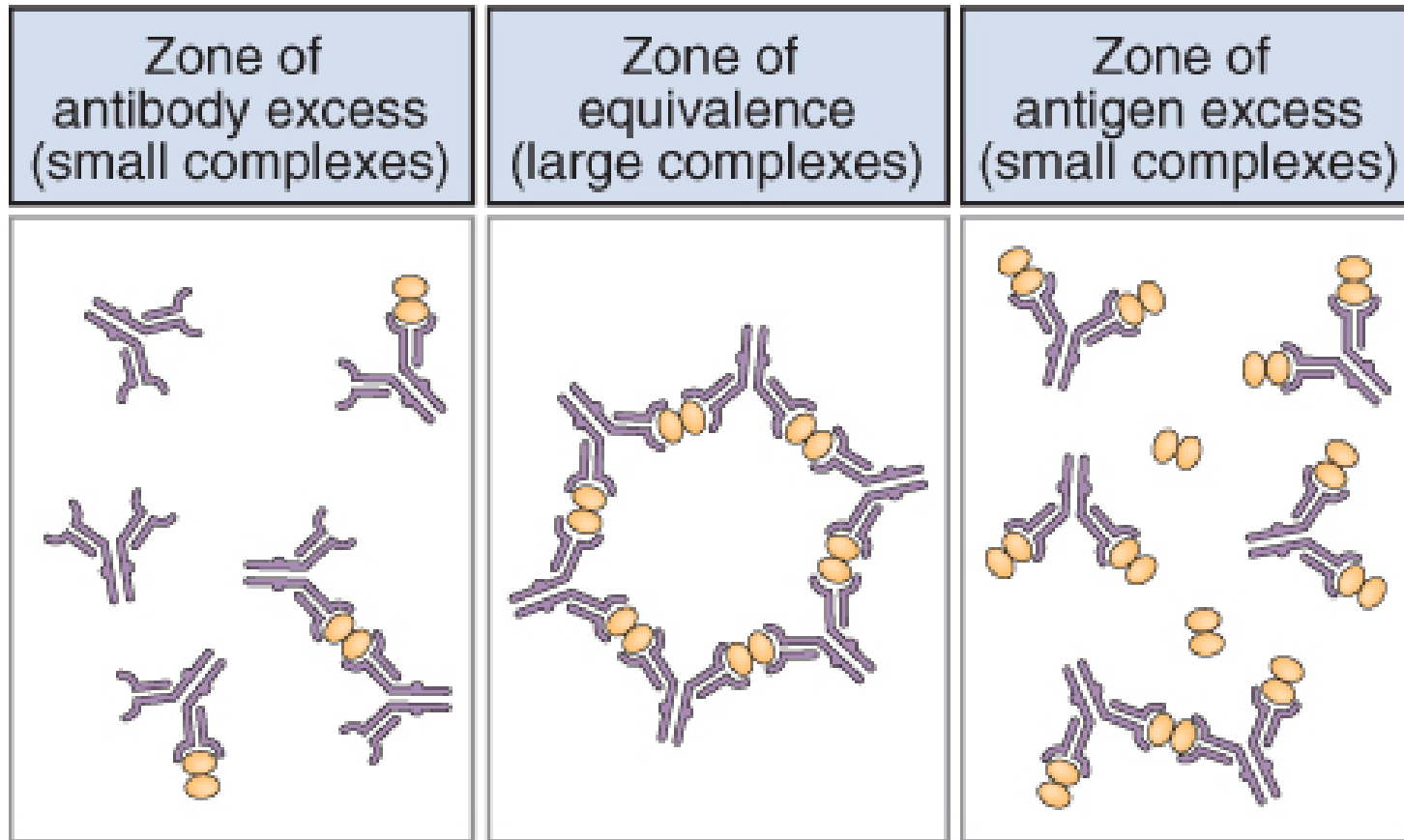
Immunodiffusion-I

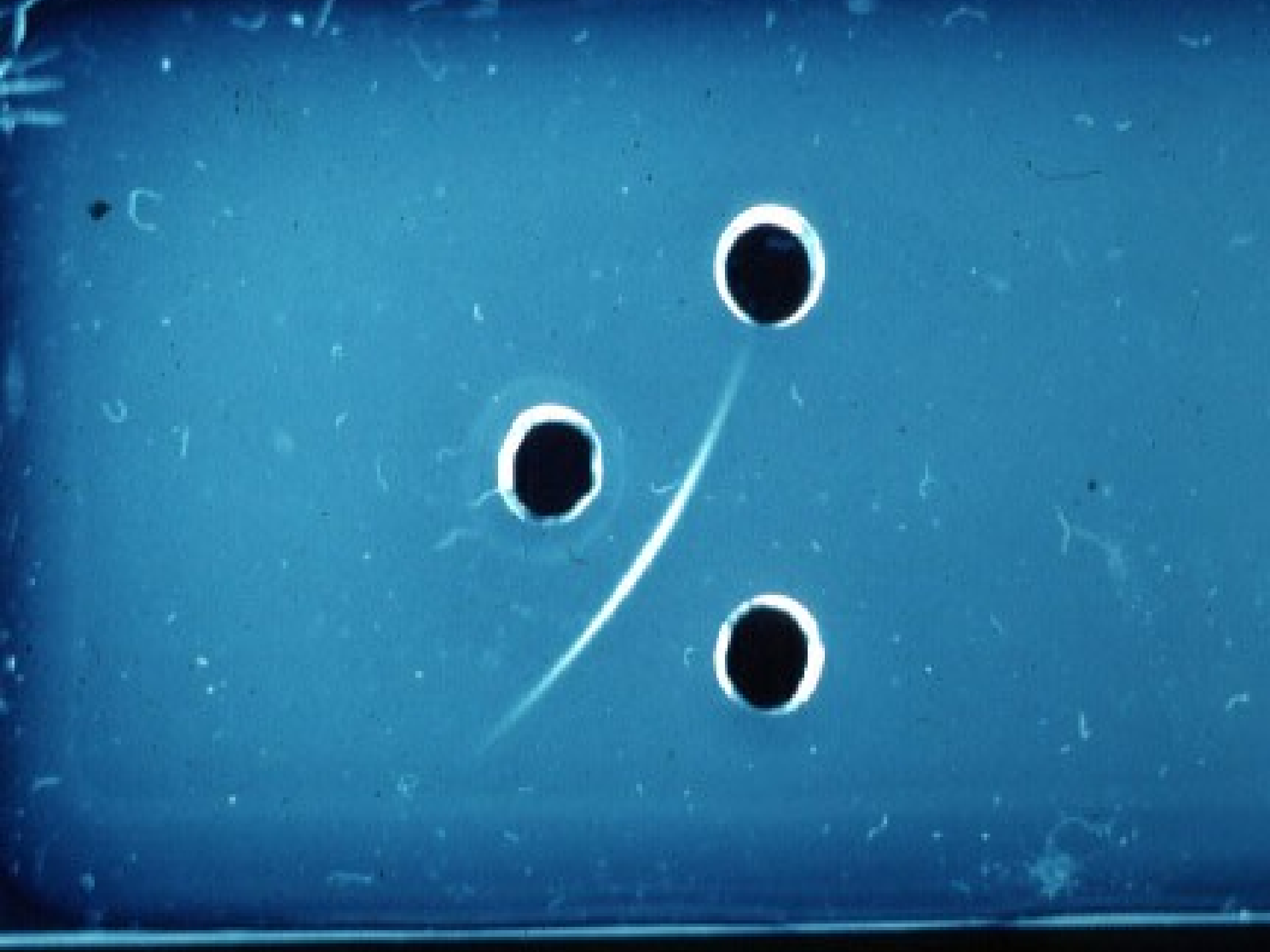


Immunodiffusion - II



Precipitate is formed only in the zone of equivalence





ELISA

ELISA

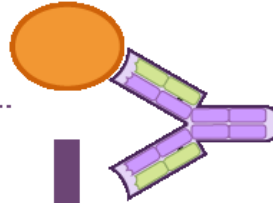
Sensitize plate with antigen ▶▶▶



Wash ▶▶▶



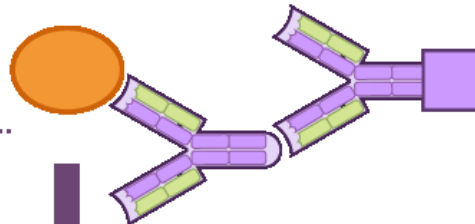
Add test antibody ▶▶▶



Wash ▶▶▶



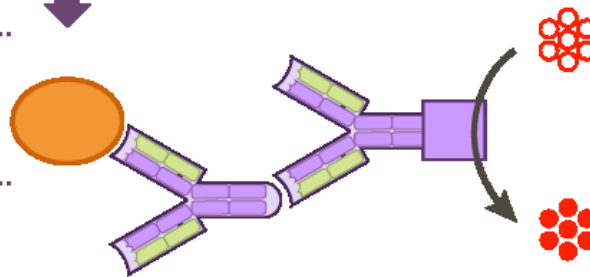
Add ligand ▶▶▶



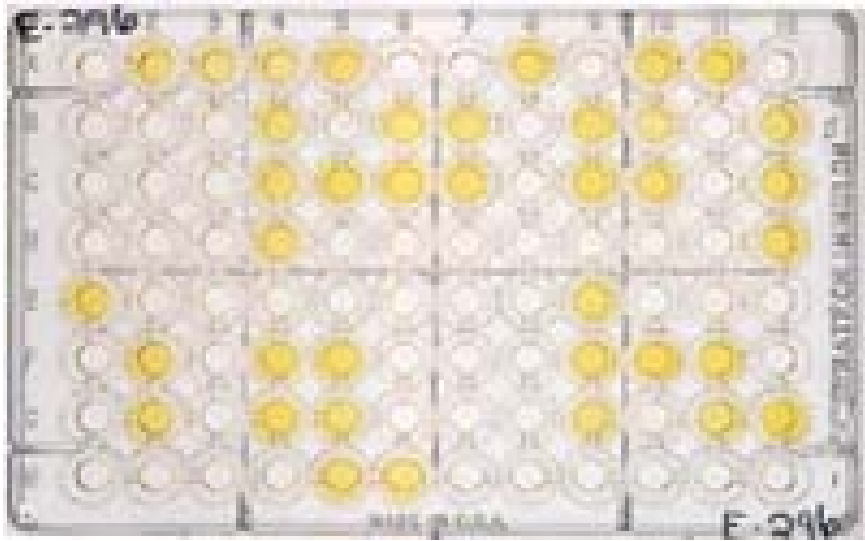
Wash ▶▶▶



Develop plate ▶▶▶



chromagen





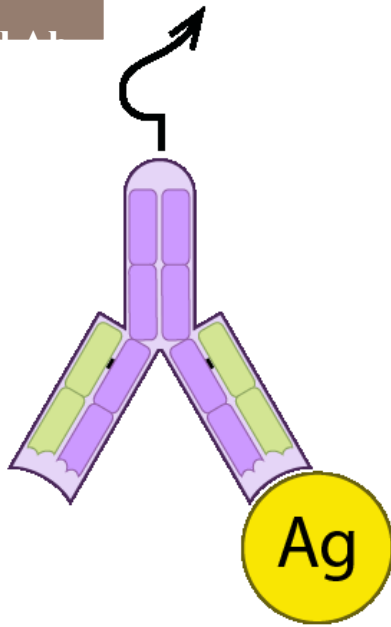
Imunofluorescence

- direct
- indirect

Imunofluorescence

DIRECT

Fluorochrome

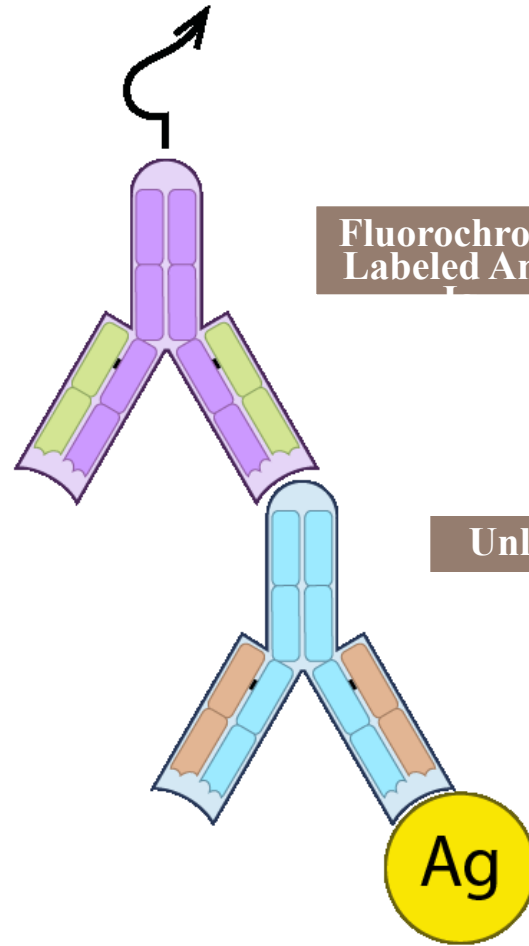


Tissue Section

INDIRECT

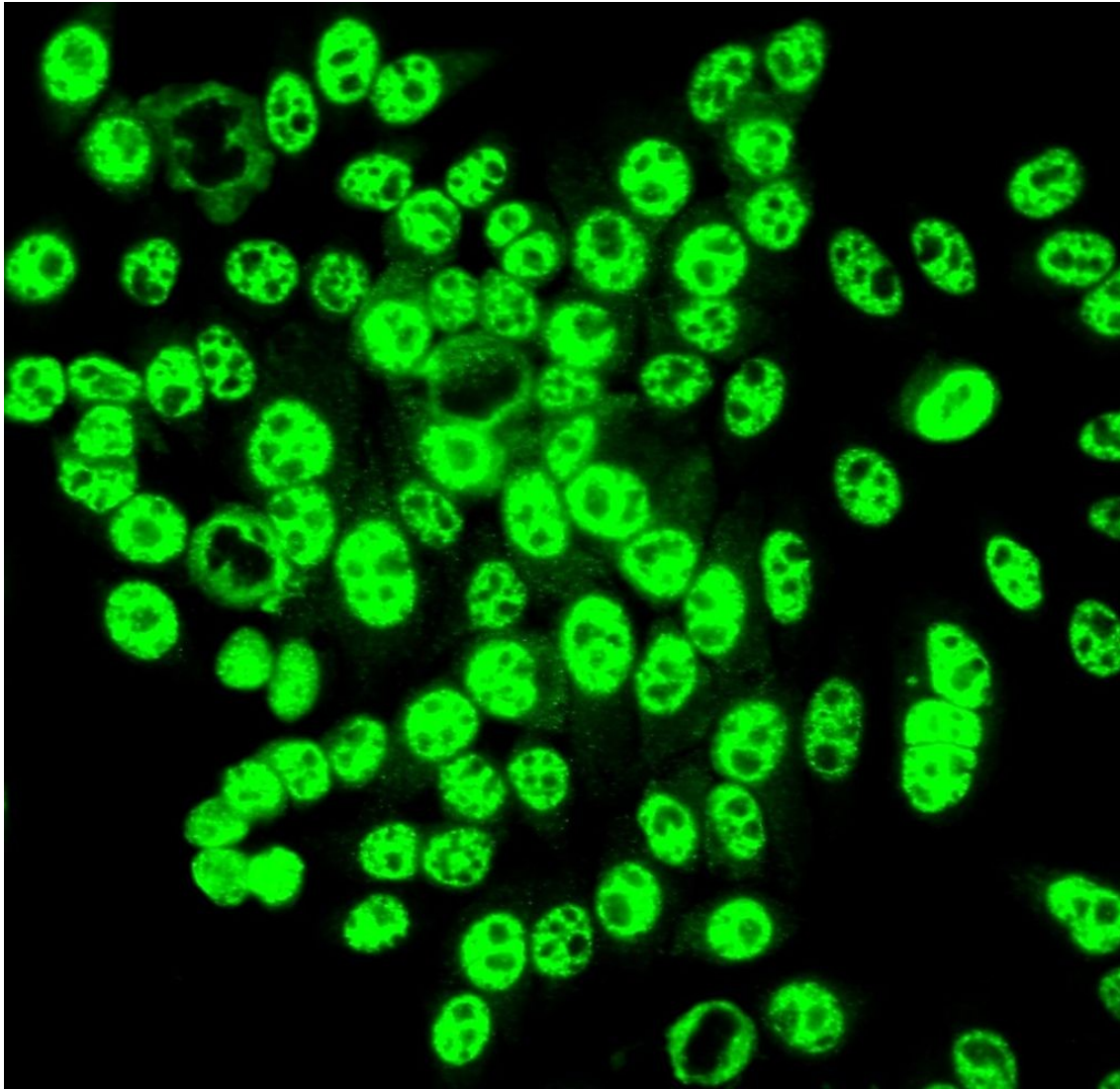
Fluorochrome Labeled Anti-

Unlabeled Ab



Tissue Section

ANA
Positive granular type



ANA – homogenous type

