

Diagnostic of infectious diseases

Direct diagnostic Visualisation

-microscopy

-antigen detection

-cultivation

-genetic material detection

Indirect diagnostic: - detection of the reaction of macroorganism to the presence of microorganism

Specific reaction of immune system

*cell immunity – skin tests

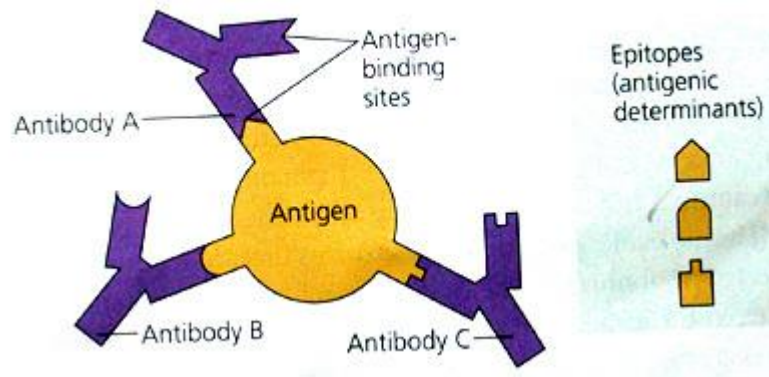
*humoral immunity – specific antibodies detection - serological reactions

Serological reactions

- are in vitro antigen-antibody reactions
- identification and quantitation of antibodies (or antigens)
- Simple serological techniques
- Specificity of antigen – determined by production of antibodies, that reacts only with it.

Antigen

- causes immune system to produce antibodies against it
- chemicals, bacteria, viruses, pollen....
- may also be formed within the body



Antigen

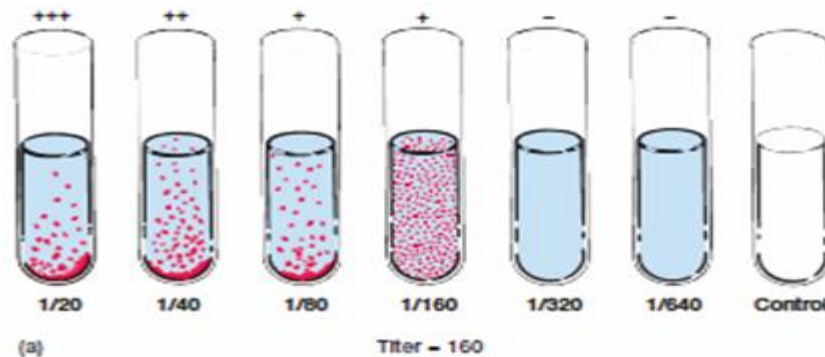
- Corpuscular
- Soluble

Antibodies

- In blood serum of immunised animals there are specific proteins – immunoglobulins, that bind antigens causing their production (Behring, Kitasato, 19th century)
- Serum – liquid that remains at the top of the tube after centrifugation of coagulated blood
- Plasma – liquid that remains at the top of tube after centrifugation of not coagulated blood

Titer

- is a way of expressing concentration
- Titer testing employs serial dilution to obtain approximate quantitative information from an analytical procedure
- The titer corresponds to the highest dilution factor that still yields a **positive reading**.



Serological reactions

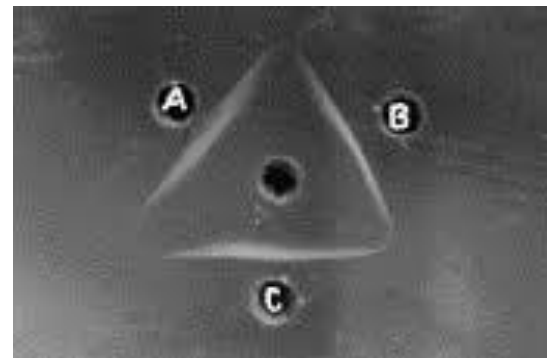
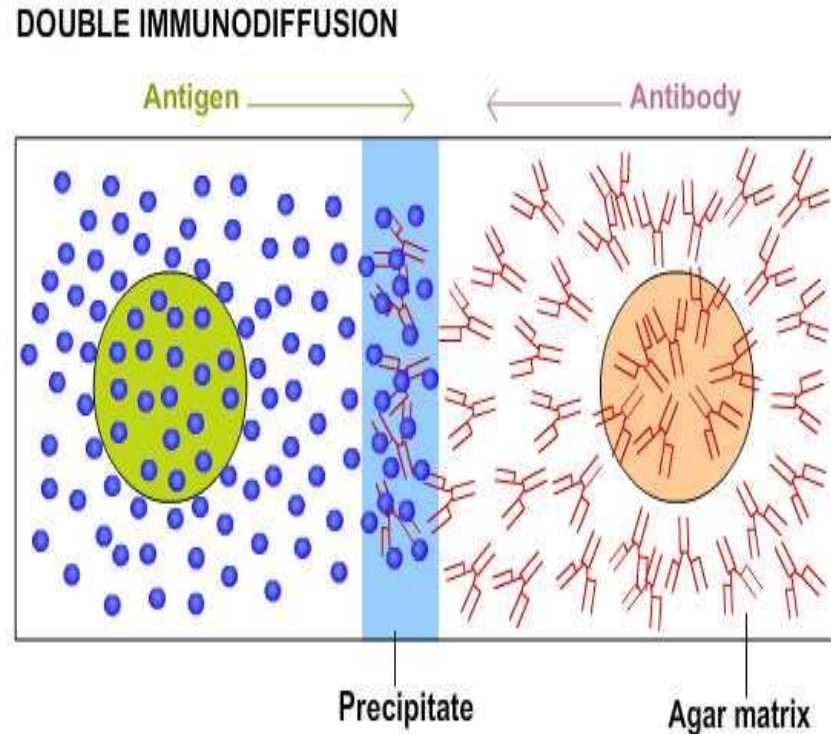
- Agglutination – antigen + dilution of serum = visible agglutination
- Latex agglutination – antibody bound on latex particles + Ag = big agglutination
- Precipitation – Ag+Ab = ring in the touch zone
- Immunodiffusion – diffusion of Ag and Ab in agar. In the meeting point – line of precipitation
- Hemagglutination – passive agglutination – ag is bound on the surface of RBC
- CF – complement fixation- Ag +Ab +C' + Ery + antieryab – lysis

Types of antigen-antibody reactions

- Reaction of antigen with specific antibody depends on the type of antigen – immunocomplexes of different quality
 - *corpuscular antigens – microorganism, erythrocytes – agglutination
 - *soluble antigens
 - small immunocomplex – in solution, in agar, -precipitation

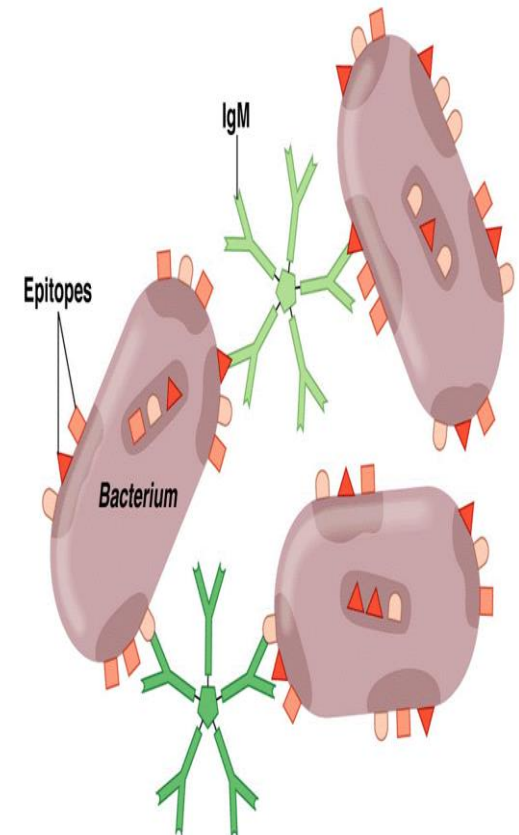
Precipitation reactions

- **Immunodiffusion** procedures are precipitation reactions carried out in an agar gel medium.
- Antibody and antigen are loaded in different wells and diffuse through the medium.
- antigen-antibody - visible band appears in the gel.



Agglutination

- The interaction of particulate antigens (cells that carry antigens) with antibodies leads to agglutination reactions.
- Diseases may be diagnosed by combining the patient's serum with a known antigen.

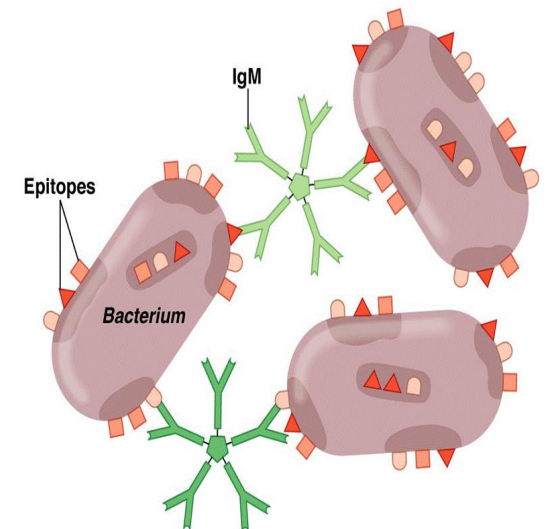


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Types of agglutination reaction

Direct agglutination.

- Corpuscular antigen-agglutinogen
- Antibody - agglutinin
- To test patient's sera (contain antibody) against large antigen.



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Figure 18.5

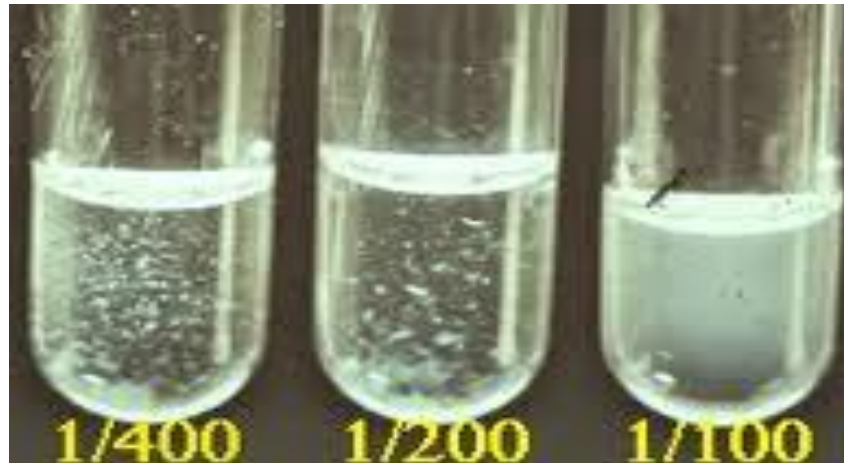
Slide agglutination

- a rapid screening test in which antibody and antigen are mixed on a glass slide and observed for agglutination



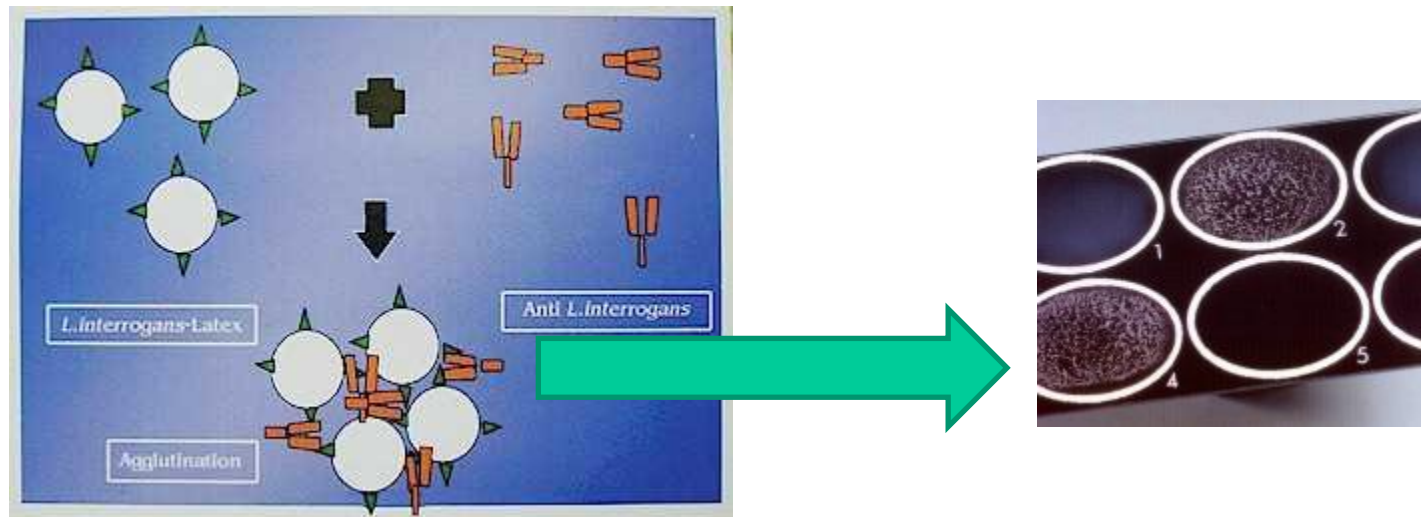
Widal reaction

- Serological agglutination test for typhoid fever (*Salmonella typhi*)
- based on the presence of agglutinating antibodies against typhoid bacteria in the patient's serum



Latex agglutination test

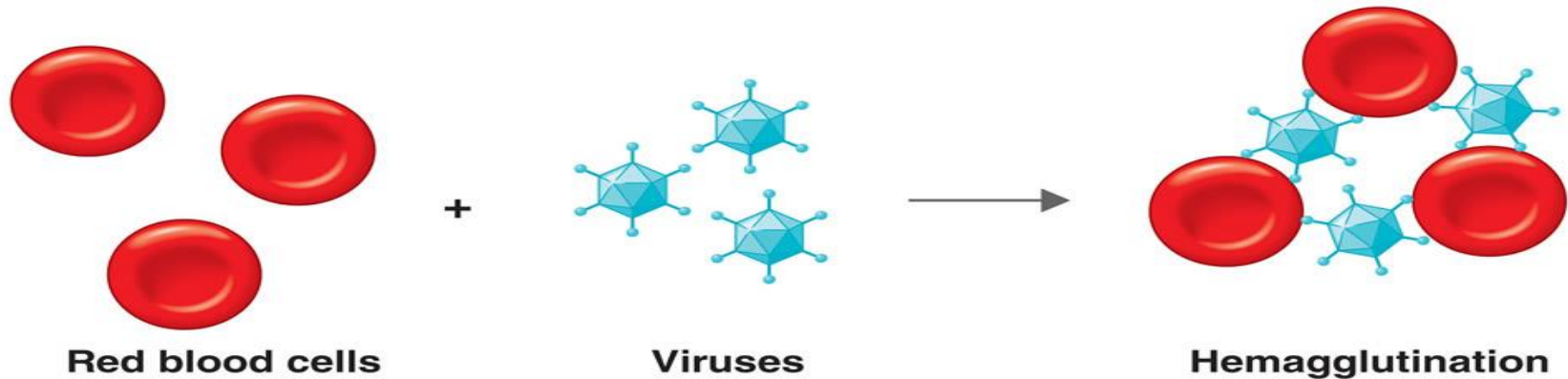
- sample is mixed with latex beads coated with antigens



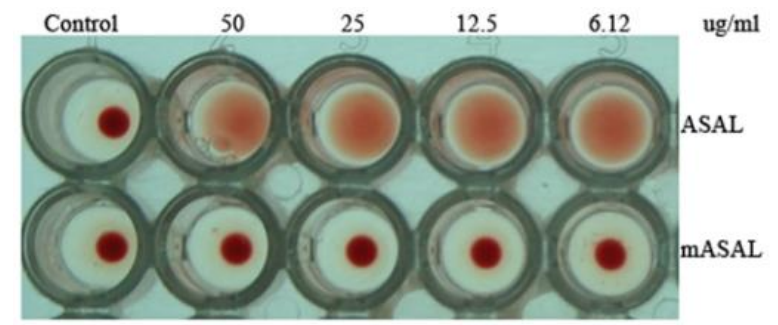
<https://www.youtube.com/watch?v=7R9og3HuAAU>

Hemagglutination

- Hemagglutination reactions involve agglutination reactions using red blood cells.
- Hemagglutination reactions are used in blood typing, the diagnosis of certain diseases, and the identification of viruses.
- Viral hemagglutination occurs when spikes on the virus cause agglutination of red blood cells - there is no antigen-antibody interaction.

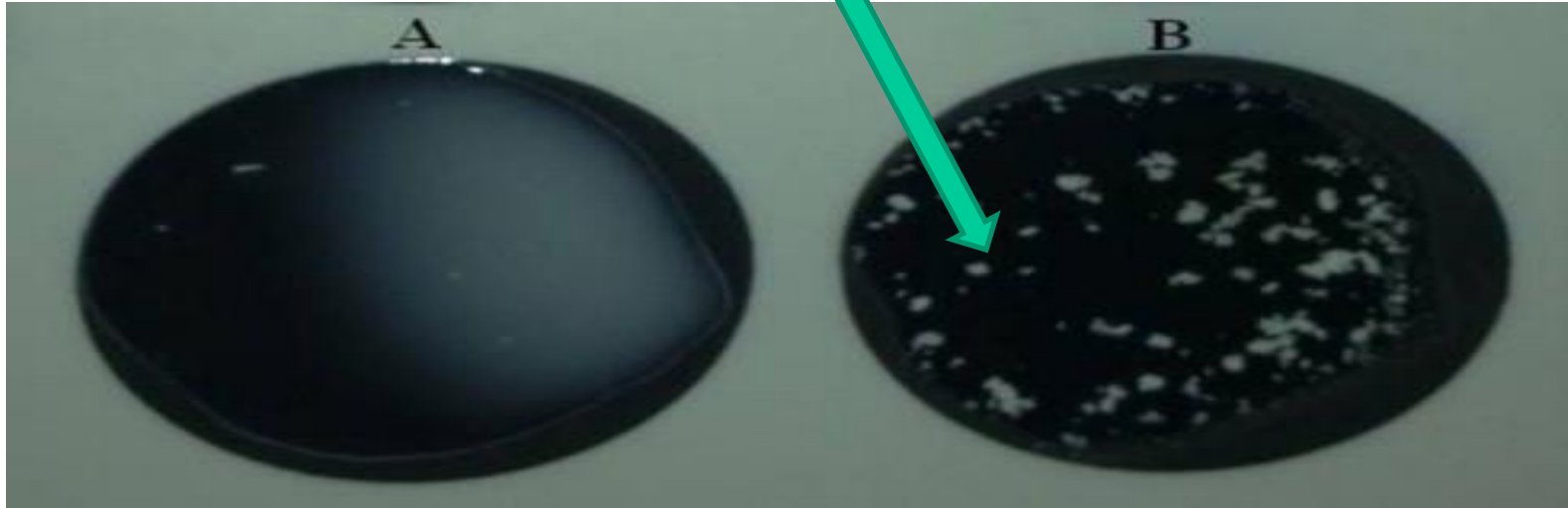


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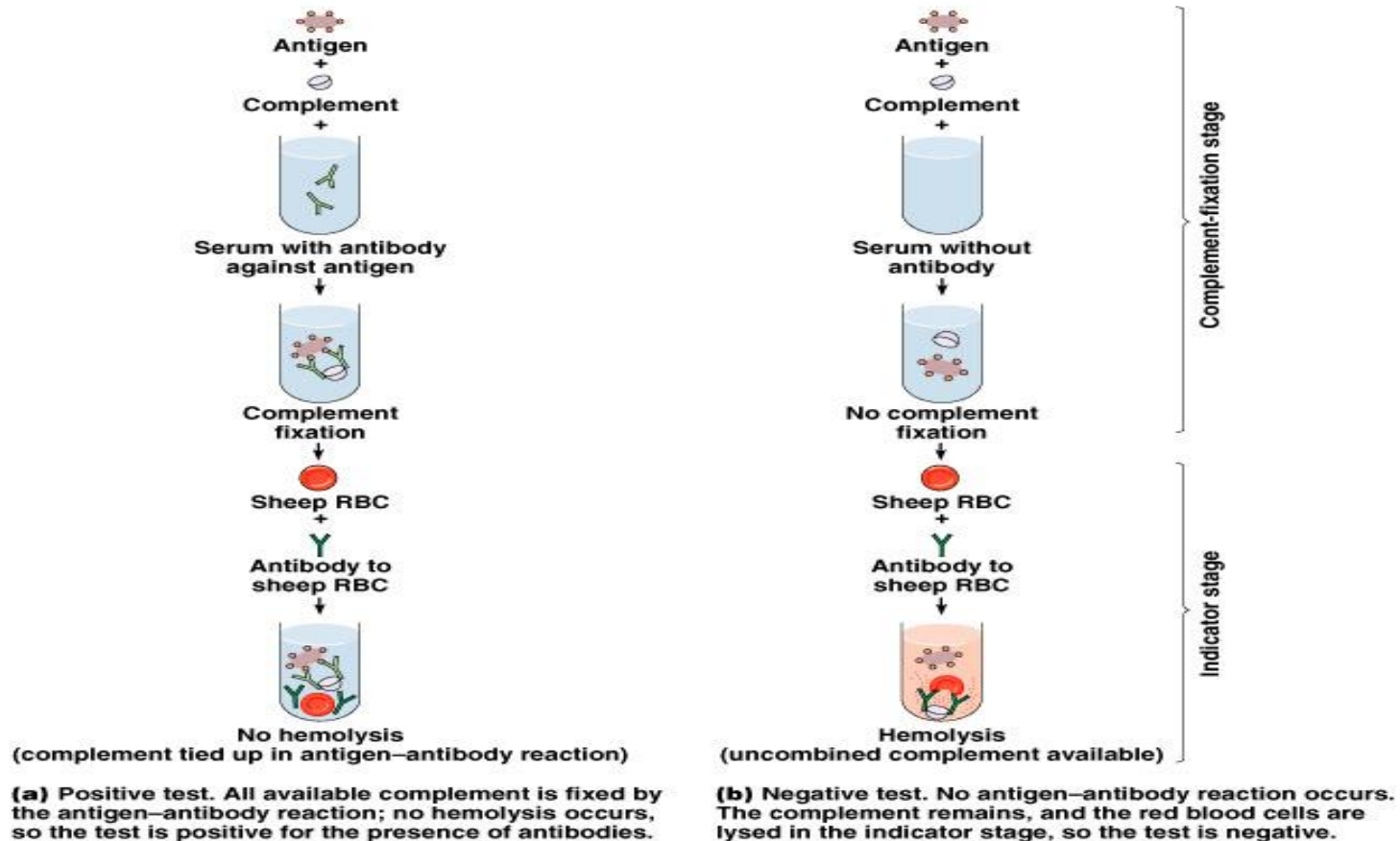


Slide indirect agglutination test (serotypization)

- **Procedure.**
 1. Put 3 drops of physiological solution on the slide.
 2. Suspend the typed bacterial colony in each drop with the bacteriological loop. The suspension looks milky opalescent.
 3. Put one loop (one drop) of anti-O diagnostic serum into one suspension and mix properly. Repeat the procedure with other diagnostic sera.
 4. The specific agglutination occurs within 2 minutes, it is visible by a naked eye.
- Agglutinated bacteria look like the grains in a clear solution.



Complement-Fixation Reactions



ELISA, RIA, IFT

- Ag + séru (Ab) + antibody against the complex Ag+Ab* (labelled with enzyme, radioactive labell or fluerescent labell)
- Detection of enzyme activity, radioactivity or fluorecence

Reading of serological reactions

- Qualitative reaction – positive or negative (comparison with bordeline value)
- Quantitative reaction – titer of antibodies, or concentration

Determination of total antibodies (CF,) – dynamics of antibody production – 2 samples in the interval of 14-21 days. Results – titer of antibodies = turned out dilution of serum

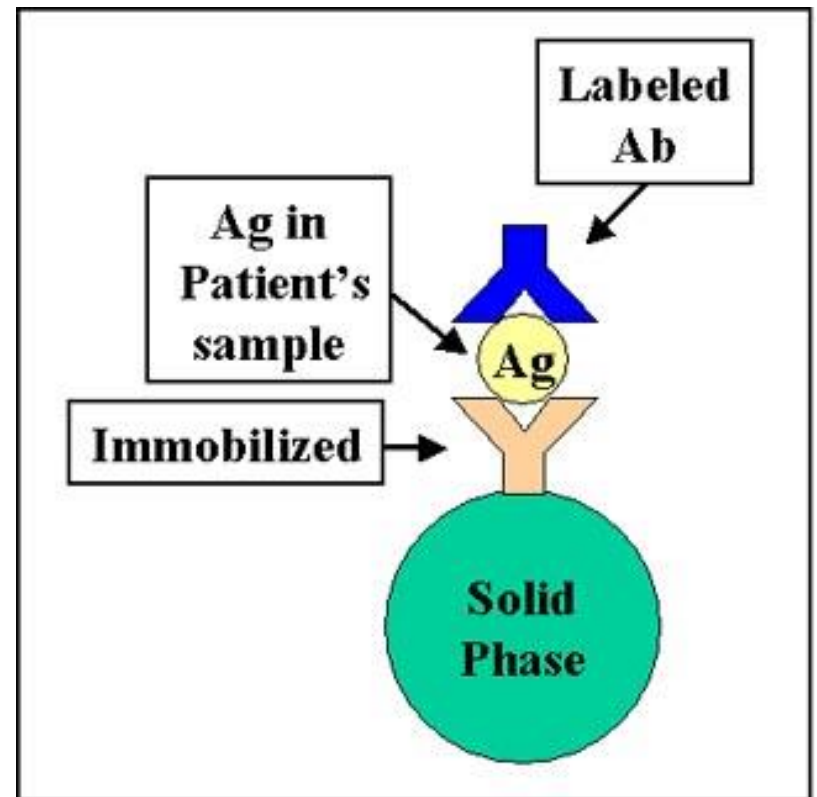
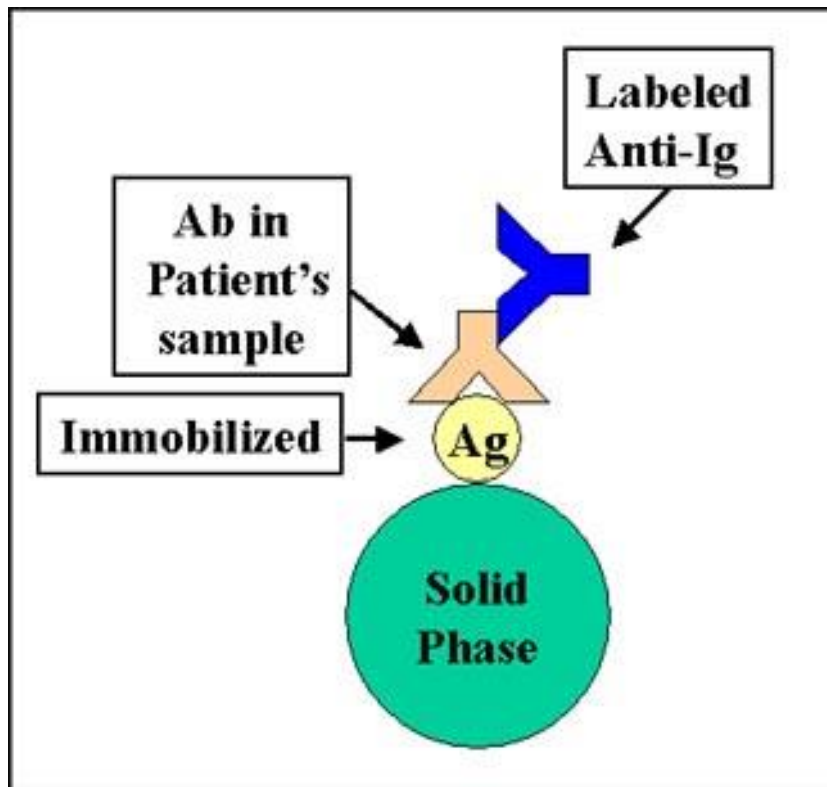
Determination of immunoglobulin classes – IgA, IgM – acute
IgG – long lasting, protective, later

Results – in concentration of antibodies – g/l

Confirmation of acute infection:

Seroconversion – from negativity to positivity, fourfold increase of total antibody titer, or presence of IgM

ELISA



immunofluorescence

