Immunology Lab 3

C-Reactive Protein (CRP)

The inflammatory marker

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Serological reactions

Serology

Is the scientific study of blood sera and their effects. It is concerned with *in-vitro* Ag-Ab reaction.

Serology as a science began in 1901. Karl Landsteiner (1868-1943) identified groups of RBCs as A, B, and O.

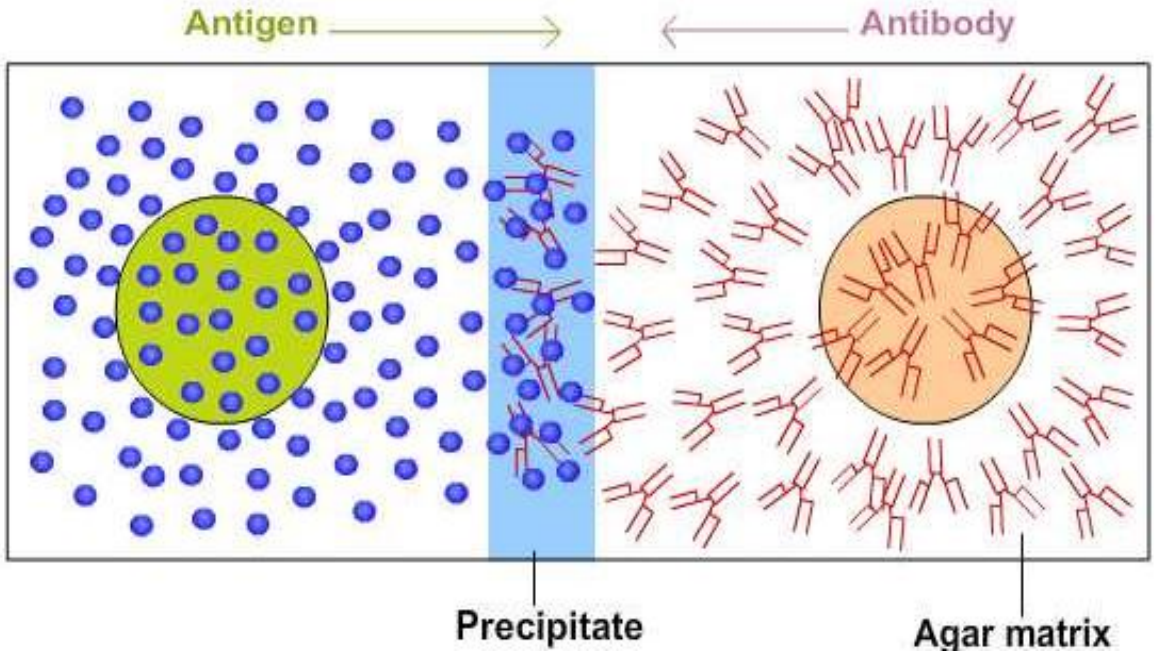
Examples of serological tests:

Ag-Ab reactions can be identified by different tests. These includes:

1. Precipitation Test: It is a type of reaction between **soluble** Ag & its specific Ab.

A- Immunodiffusion

B- Immunoelectrophoresis



2. **Agglutination Test**: Agglutination occurs due to the cross-linking of Abs with particulate Ags.

Types of Agglutination

a. **Direct agglutination:** This reaction is between **insoluble** Ag & its specific Ab. Example: Bacterial agglutination.

b. **Passive (indirect) Latex-agglutination:** When the test Ag is soluble, it needs to use a carrier so that the carrier will be agglutinated in the presence of a specific Ab, producing a reaction that is easy to see.

Examples of carriers are latex particles, and gelatin.

Mechanisms of Agglutination:

Occur in two stages:

- **Sensitization**, physical attachment of Ag & Ab.
- **Network formation**.

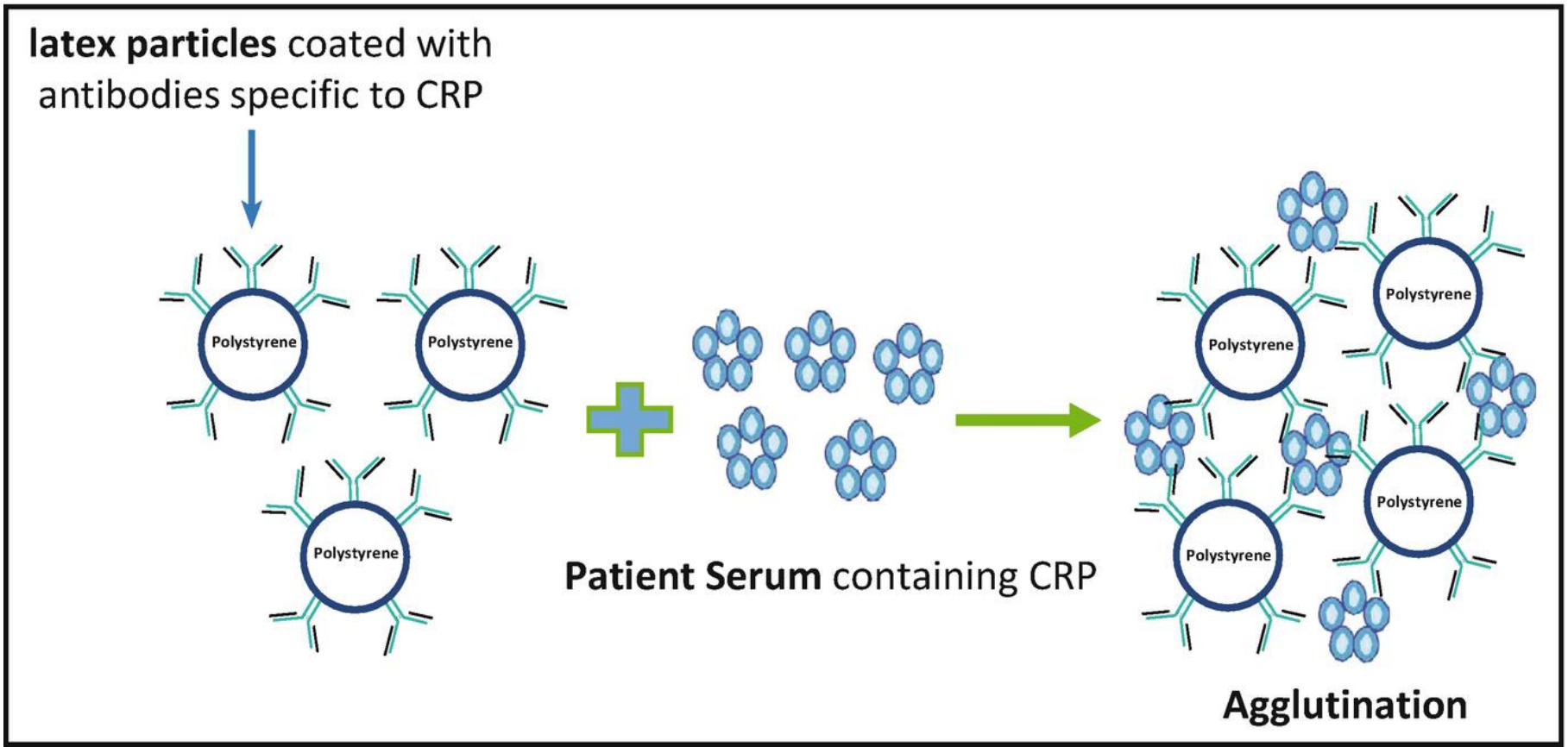
Example of indirect Agglutination:

Latex Agglutination

a) Ab or Ag molecules are bound to the surface of latex carrier particles.

b) If Ag or Ab is present in the sample, cross-linking will occur & visible aggregates.

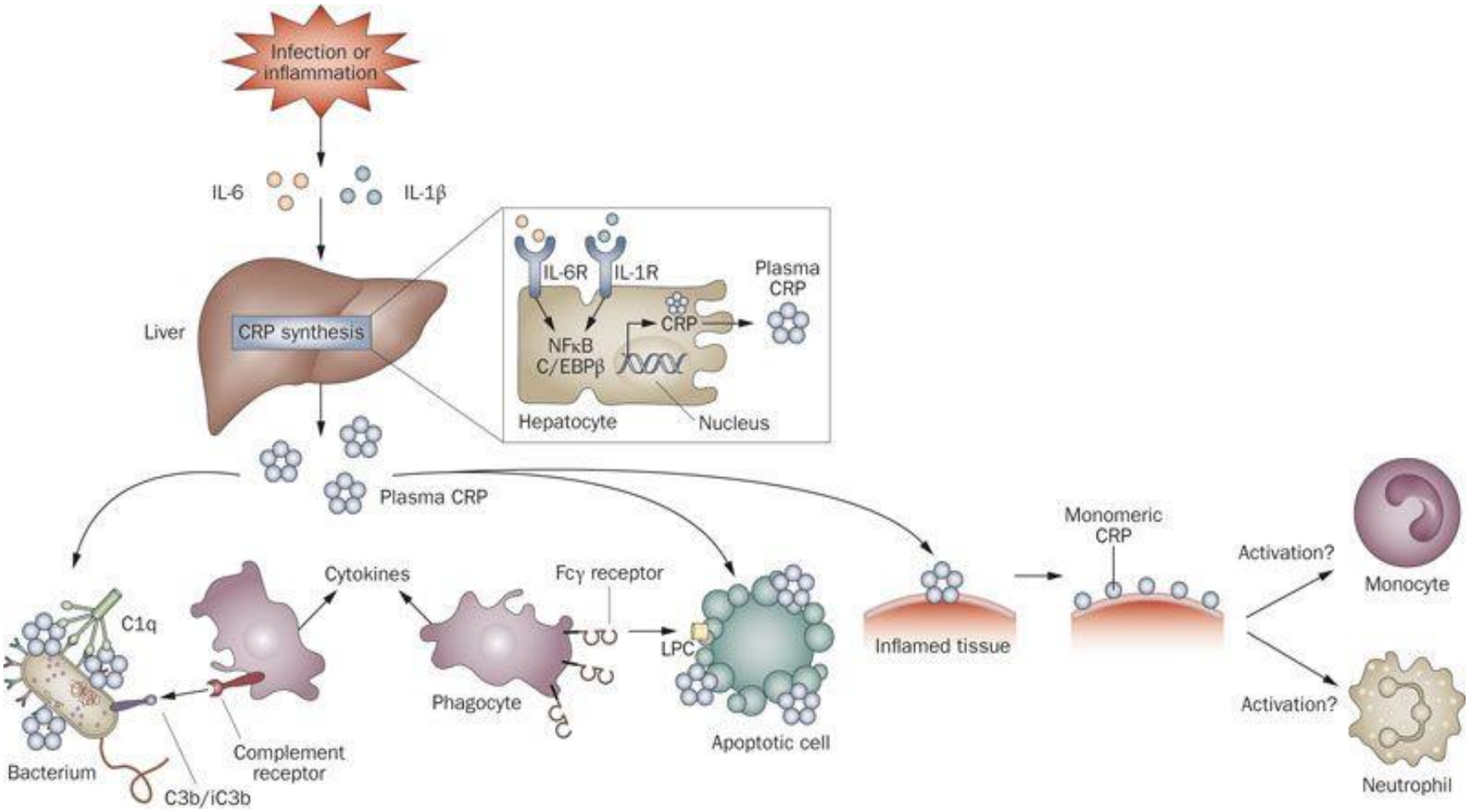
Examples: C-reactive protein (CRP), Rheumatoid Arthritis (RA), etc.

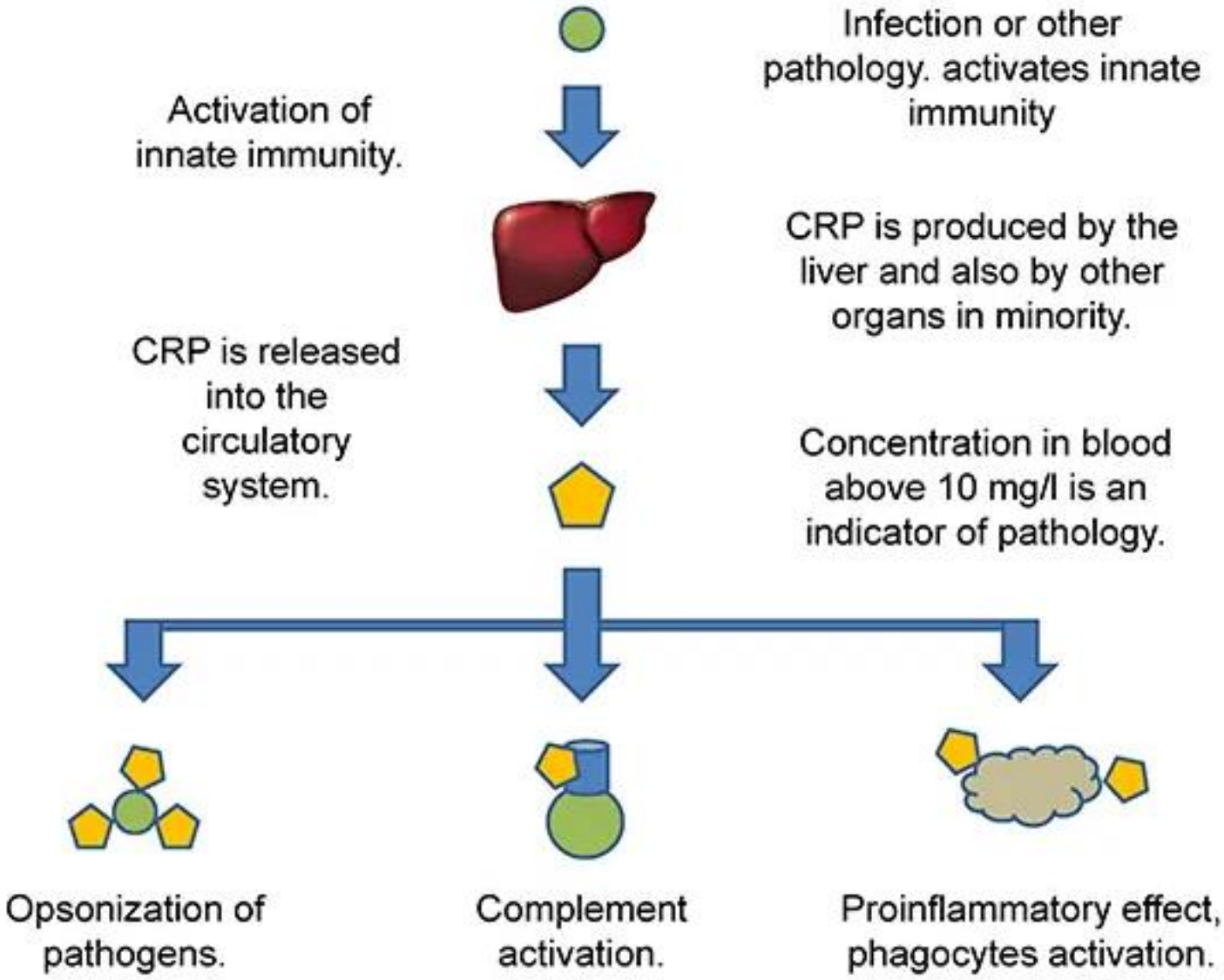


C-Reactive Protein (CRP)

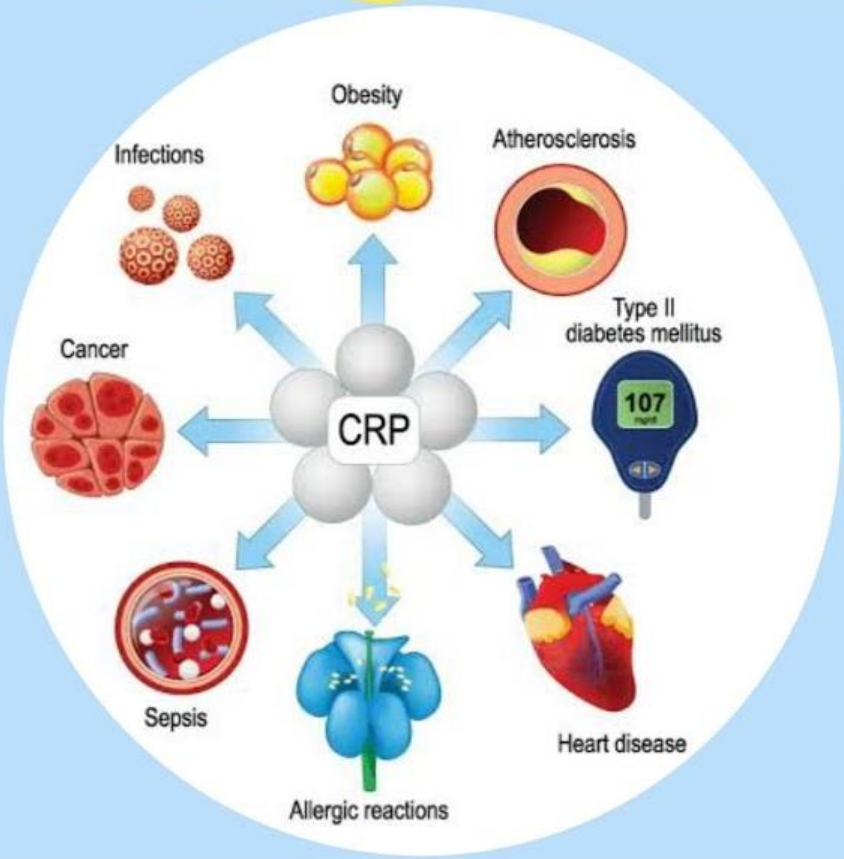
- C-reactive protein (CRP) is a protein found in the blood produced by the liver in response to inflammation.
- Is one of the acute phase proteins, which is used to **detect acute inflammatory diseases**.
- CRP was first identified in the serum of patients with pneumonia because it precipitated with the **C-polysaccharide** on the **Pneumococcal** cell wall.
- CRP activates complement by binding C1q and initiating the classical pathway.
- Opsonize particles for phagocytosis.
- CRP increases rapidly in serum within (4-6) hrs of the onset of inflammation (infection), reaches a peak within 48 hrs, and declines rapidly with a recovery of inflammation.

Synthesis and functions of CRP




Infection or other pathology. activates innate immunity
Activation of innate immunity.
CRP is produced by the liver and also by other organs in minority.
CRP is released into the circulatory system.
Concentration in blood above 10 mg/l is an indicator of pathology.
Opsonization of pathogens.
Complement activation.
Proinflammatory effect, phagocytes activation.

High CRP Symptoms



You may need CRP test if you have symptoms like:

Fever

Chills

Rapid breathing

Rapid heart rate

Principle

- The C-reactive protein test is done to detect acute inflammations and to estimate the qualitative and semi-quantitative CRP in human serum samples. The normal range is less than **6mg/L**.
- The reagent contains latex particles coated with anti-human CRP antibodies. Agglutination occurs in the presence of CRP in the patient's serum.

Procedures

Qualitative:

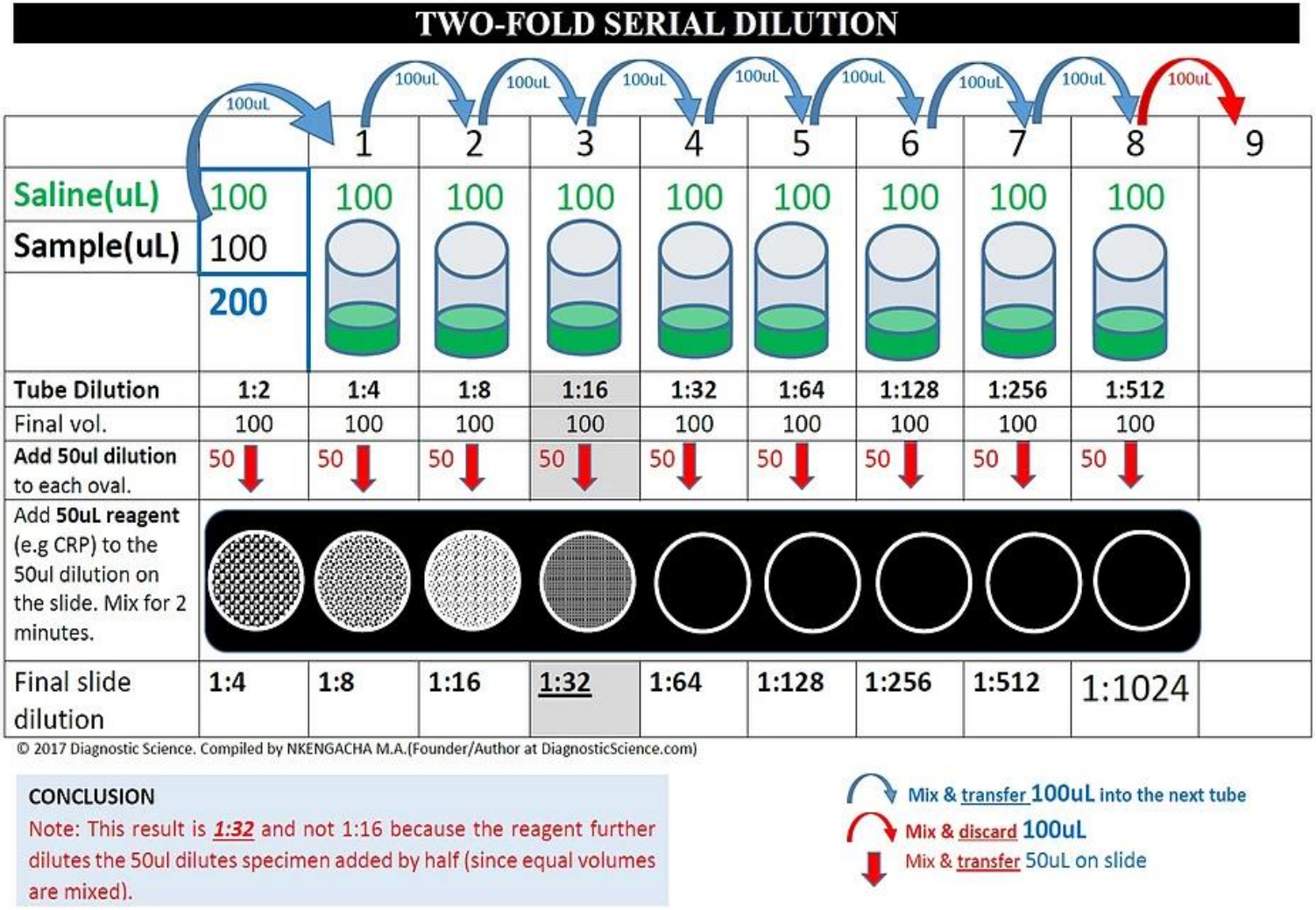
1. Allow each component to reach room temperature.
2. Gently shake the latex reagent to disperse the particles.
3. Add one drop of serum on the black circle test slide, using the disposable pipettes provided.
4. Add one drop of CRP latex reagent next to the drop of serum.
5. Mix both drops with a stirrer and spread over the entire area of the test circle.
6. Tilt or rotate the slide backward and forward for 2 minutes.
7. Look for agglutination.
8. Interpret results immediately after 2 minutes.

Results

- **+Ve result**: The presence of agglutination indicates a level of CRP in the sample ≥ 6mg/L.
- **-Ve result**: no agglutination indicates a level of CRP in the sample < **6mg/L**.

Semi-quantitative:

This method can be performed in the same way as the qualitative test, using dilution of the serum in normal saline as below:

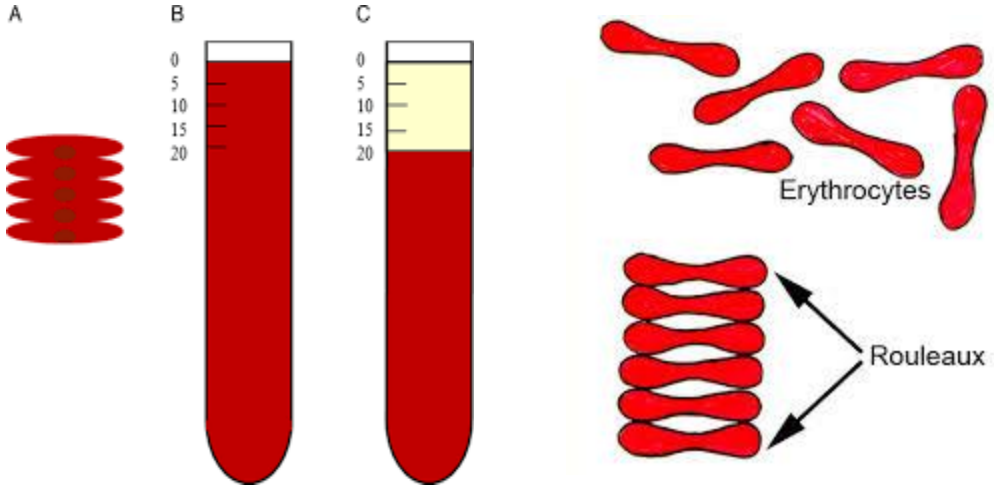


Result:

The serum titer is examined as the reciprocal of the **highest dilution** showing macroscopic agglutination, e.g. If this occurs in dilution 4, the result is 1:32.

Erythrocyte Sedimentation Rate (ESR)

- ESR is a type of blood test that measures how quickly erythrocytes (red blood cells) settle at the bottom of a test tube.



Age group	ESR (mm/hour)
Adults (< 50 years)	
Men	< 15
Women	< 20
Adults (> 50 years)	
Men	≥ 20
Women	≥ 30

ESR

- Non-specific test as indicative of inflammation.
- Other names: ESR, sedimentation rate test; Westergren sedimentation rate
- It is used as an initial screening tool and as a follow-up test to monitor therapy and the progression or remission of disease.
- Easy to perform.
- Inexpensive.
- Unit -measured in mm/hr.