Antigen-Antibody

reactions (2)

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Learning **objectives:**

- introduction to Antigen Antibody reactions**.**
- **Antigen Antibody reactions part1:** Precipitation, Flocculation and Immunodiffusion.
- **Antigen Antibody reactions part 2:** Agglutination.
- **Antigen Antibody reactions part 3:** Complement Fixation Test.

2.

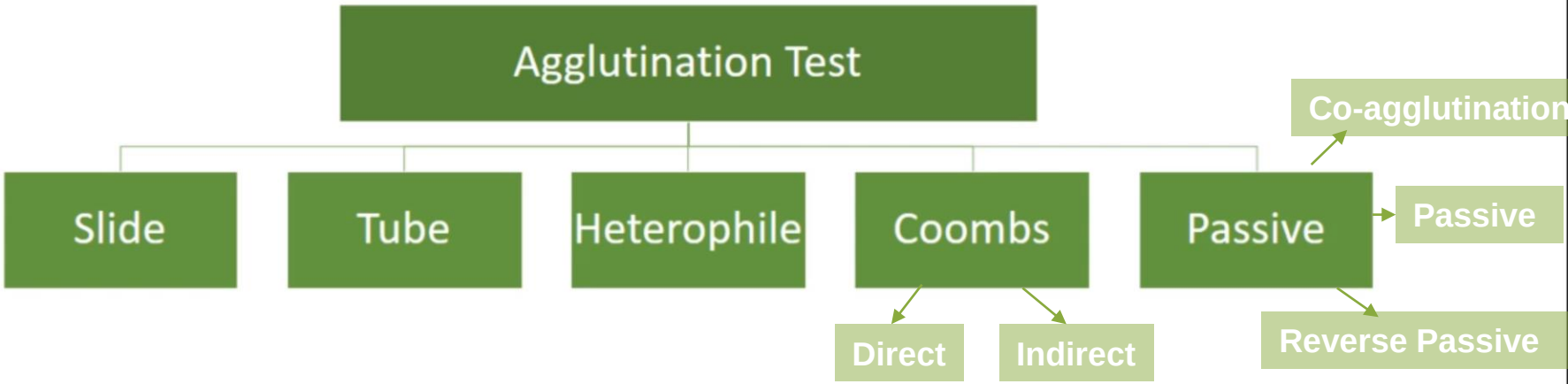
Agglutination

AGGLUTINATION

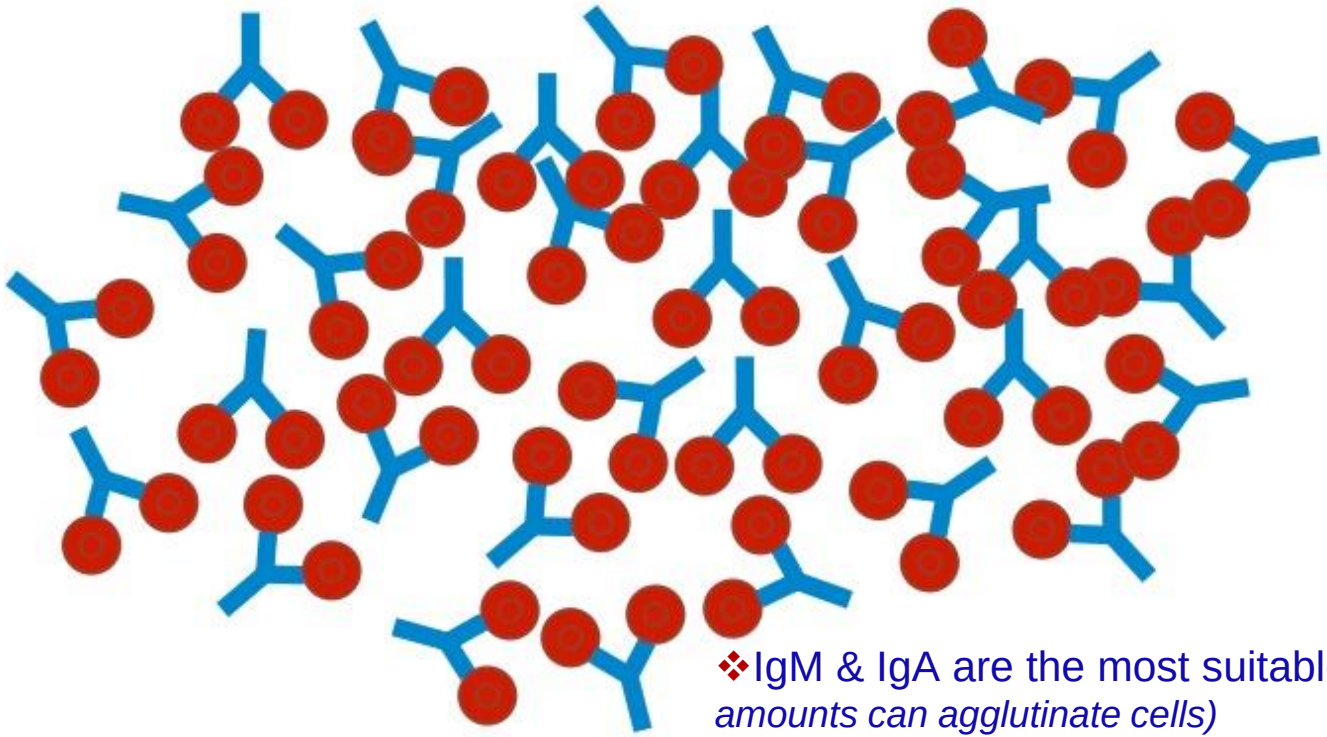
Particulate antigen + Antibody + Electrolytes - Suitable temperature → detected by agglutination (**clumping**) of the antigen.

Agglutination optimal - Ag + Ab - **Equivalence** →

As in Precipitation Agglutination depend on **(lattice theory)**



Agglutination: The action of an antibody when it cross-links multiple antigens producing clumps of antigens



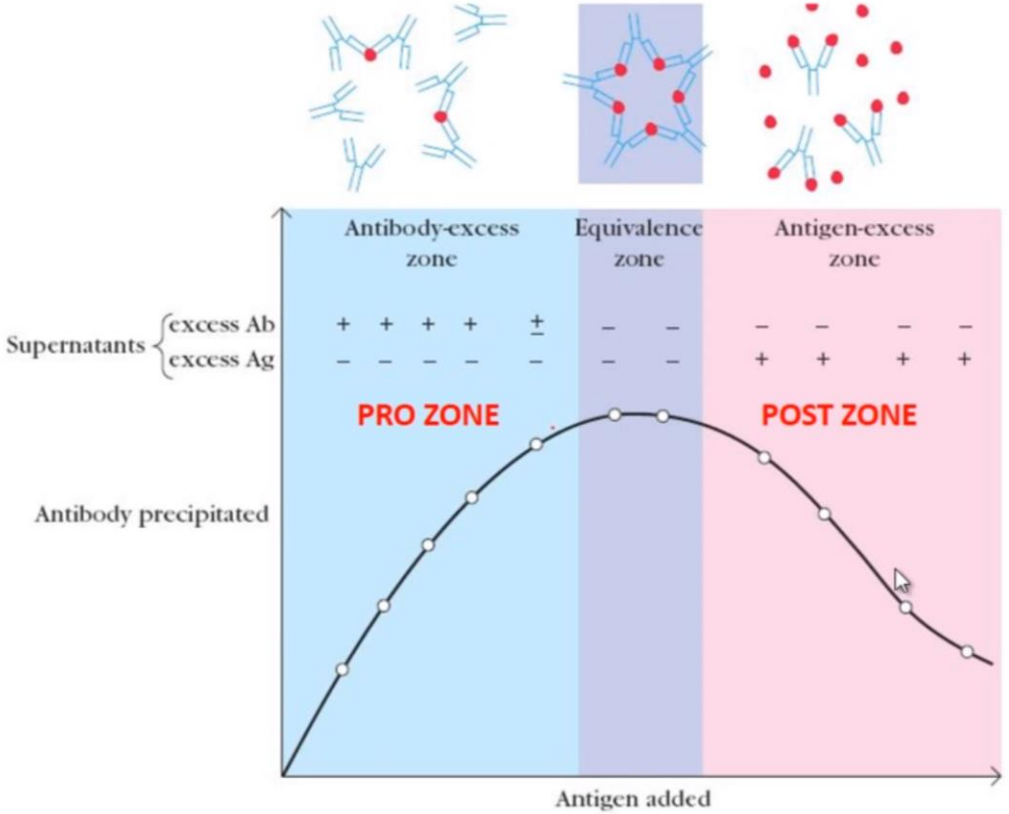
❖IgM & IgA are the most suitable *(IgG in sufficient amounts can agglutinate cells)*

LATTICE THEORY

Zone of antibody excess (Prozone)
precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant

Zone equivalence
Maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant;

Zone of antigen excess (Postzone)
Precipitation is inhibited & Ag. not bound to Ab. can be detected in the supernatant



Why do we need Agglutination if we have Perception?!

1. Agglutination is more sensitive compered to perception.
2. Clumps are better for visualization.
3. Faster.

1- SLIDE AGGLUTINATION

(Qualitative)

- Uniform suspension of particulate antigen and appropriate antiserum
- Positive result - Clumping of particles and clearing of the drop

e.g.

1. Typing of bacterial isolates
2. Blood groups and typing

Blood Types	Antigens Present ▲ = A ● = B ● = D	Antibodies Present Anti-A Anti-B Anti-D	Blood Typing Anti-A Anti-B Anti-D	Can Receive Blood From... (or, Can Donate Plasma To)	Can Donate Blood To... (or, Can Receive Plasma From)
A^+		Anti-B		A^+ A^- O^+ O^-	A^+ AB^+
A^-		Anti-B Anti-D		A^- O^-	A^- A^+ AB^+ AB^-
B^+		Anti-A		B^+ B^- O^+ O^-	B^+ AB^+
B^-		Anti-A Anti-D		B^- O^-	B^+ B^- AB^+ AB^-
AB^+ Universal Acceptor				A^+ A^- B^+ B^- AB^+ AB^- O^+ O^-	AB^+
AB^-		Anti-D		A^- AB^- O^- B^-	AB^+ AB^-
O^+		Anti-A Anti-B		O^+ O^-	A^+ B^+ AB^+ O^+
O^- Universal Donor		Anti-A Anti-B Anti-D		O^-	A^+ A^- B^+ B^- AB^+ AB^- O^+ O^-

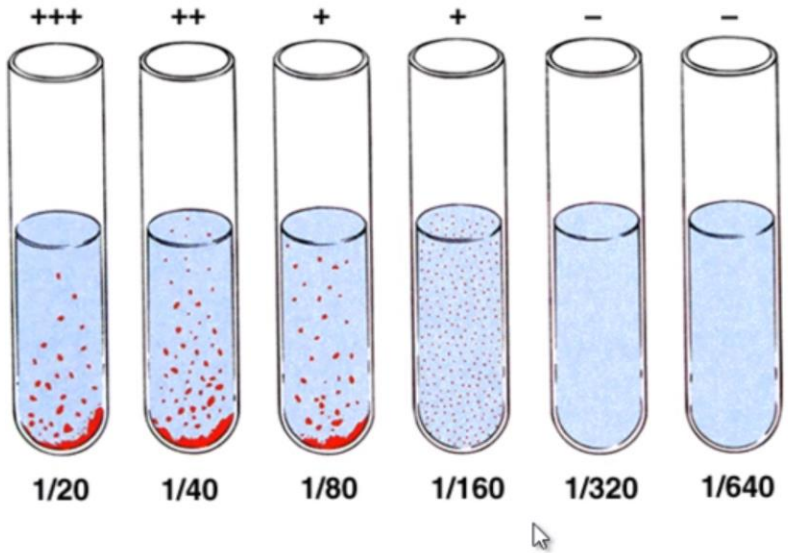
2- TUBE AGGLUTINATION

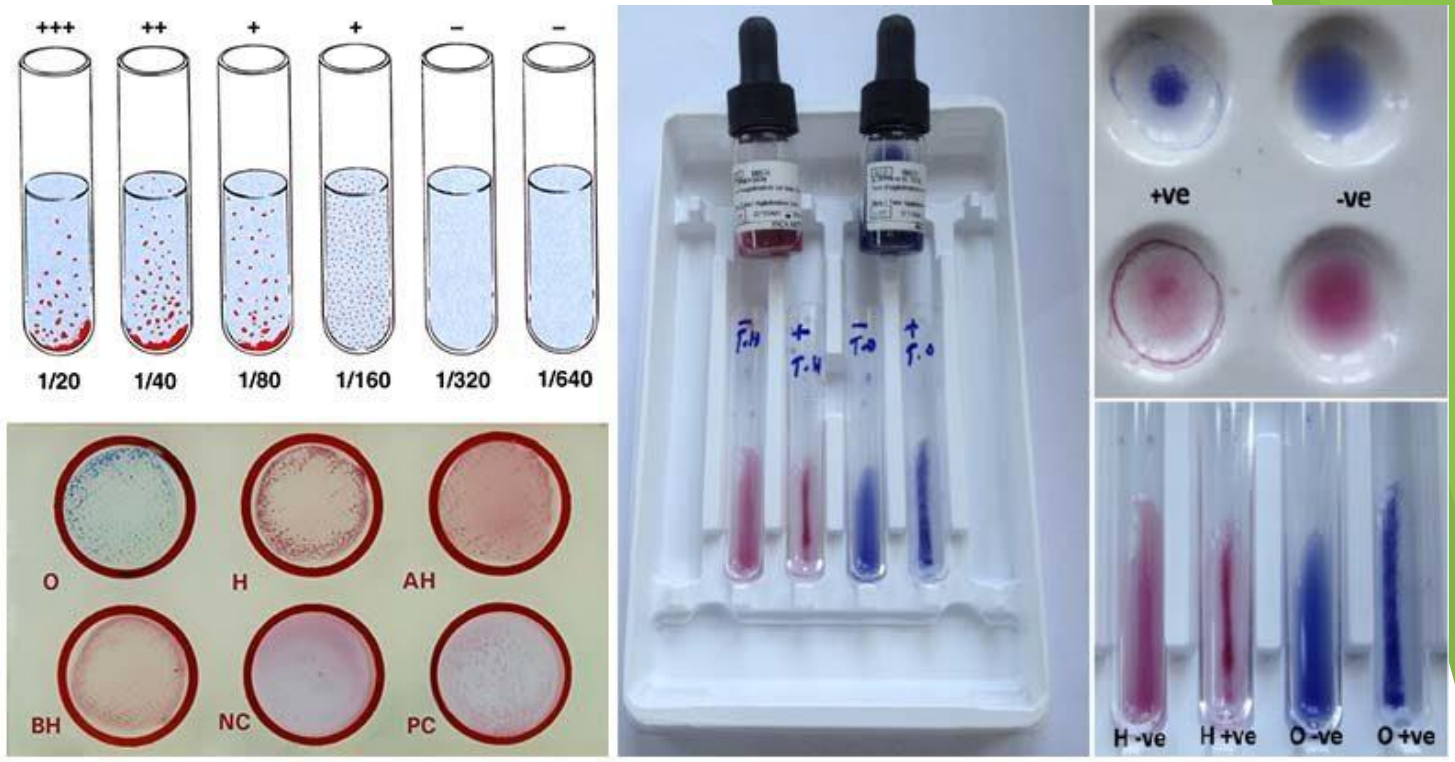
(Semi-quantitative)

- Standard quantitative method
- Particulate antigen and equal volume of serial dilution of antiserum
- Agglutination titre

(the highest dilution that shows a reaction)

e.g.
- Diagnosis – Typhoid (Widal), brucellosis, typhus fever



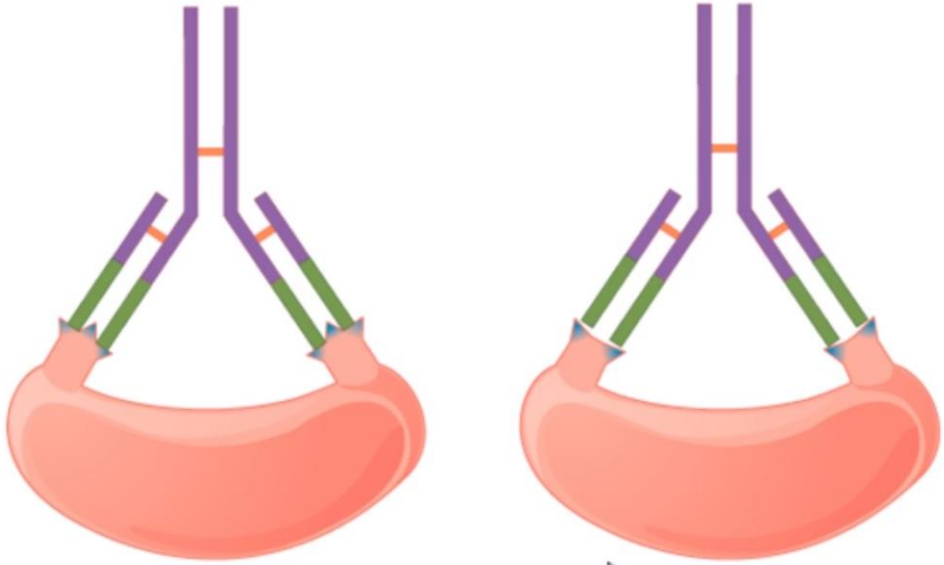


Widal test: detects the presence of antibodies in patient's serum produced against the causative agents of enteric fever (*Salmonella* Typhi and *Salmonella* Paratyphi A, B and C). O antigen on the cell wall and H antigen on flagella.

3- HETEROPHILE AGGLUTINATION

(in case the targeted Ag is challenging to get)

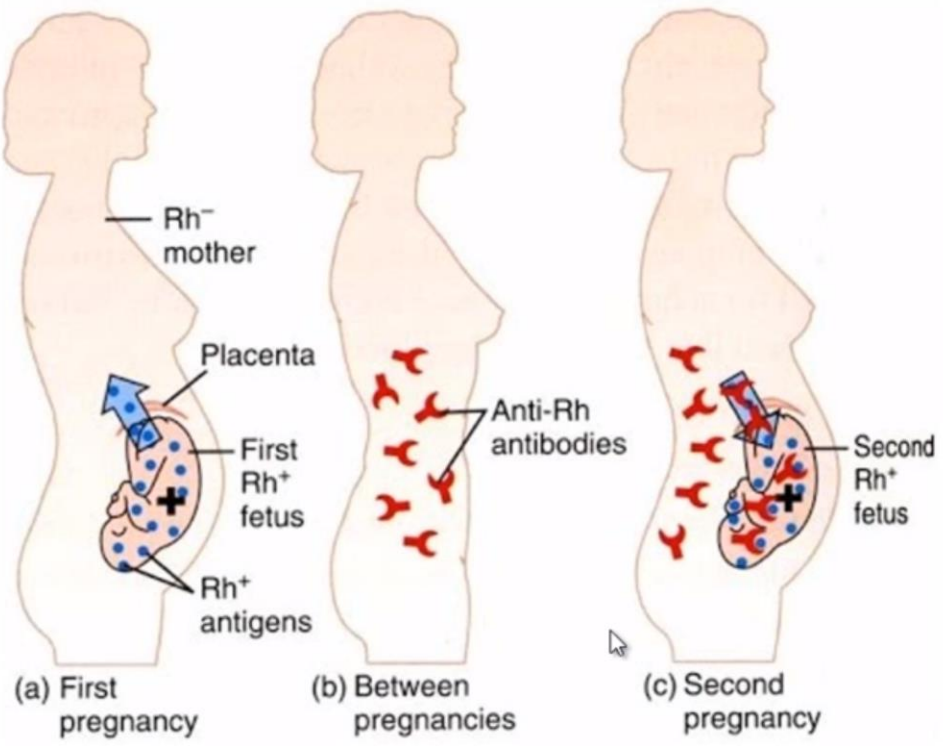
Test	Want to detect Ab against	Use cross reacting Ag of
Weil felix reaction	Ricketssiae (typhus fever)	Proteus
Paul bunnel test	EBV (infectious mononucleosis)	OX RBC
Streptococcus MG agglutination test	Mycoplasma (atypical pneumonia)	Streptococcus MG



An antibody may react with two different epitopes.

4- ANTIGLOBULIN (COOMBS) TEST

- **Erythroblastosis fetalis diagnosis:**
- **Detection of Rh antibody in**
  - **Mothers serum**
  - **Fetal RBC**
- **Incomplete** Rh **antibody** coats the surface of Rh+ RBCs but not cause agglutination.
- Rabbit antibody against human gamma globulin (**Coomb's serum**) is used.



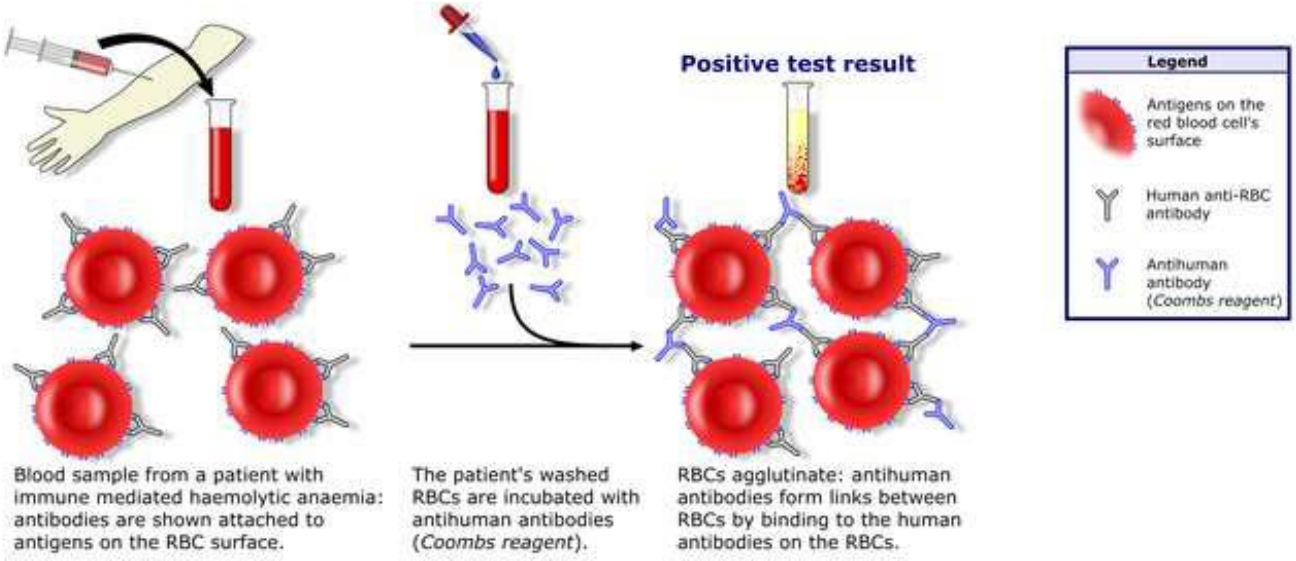
(a) Direct Coomb's Test

When antibodies bind to erythrocytes, they do not always result in agglutination. This can result from the antigen/antibody ratio being in antigen excess or antibody excess or in

These antibodies that bind to but **do not cause agglutination** of red blood cells are sometimes referred to as **incomplete antibodies**.

In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds

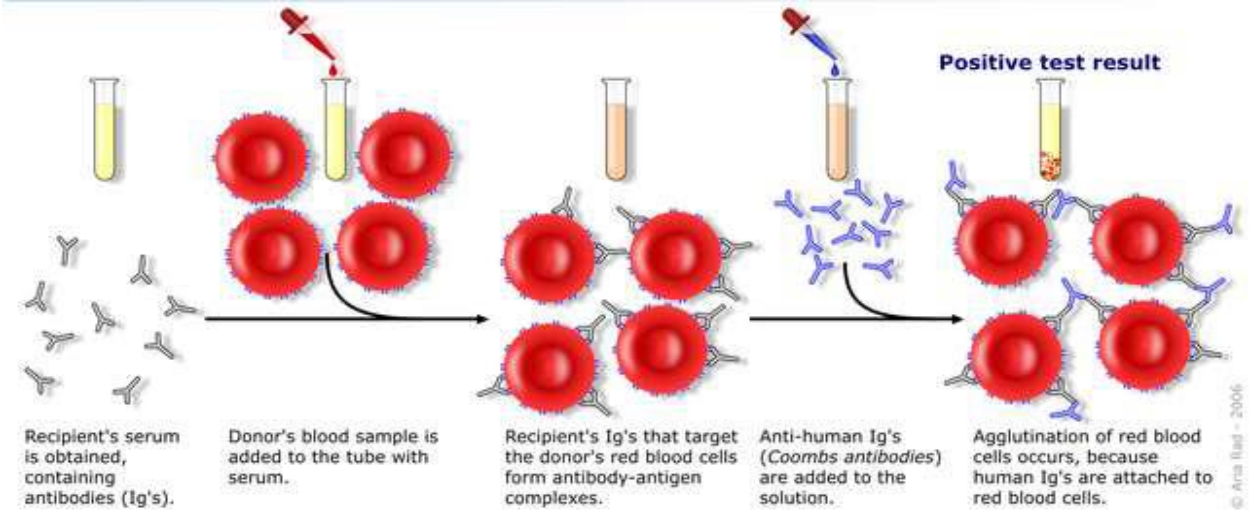
a second antibody directed against the immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can now cross link the red blood cells and result in agglutination.



(b) Indirect Coomb's Test

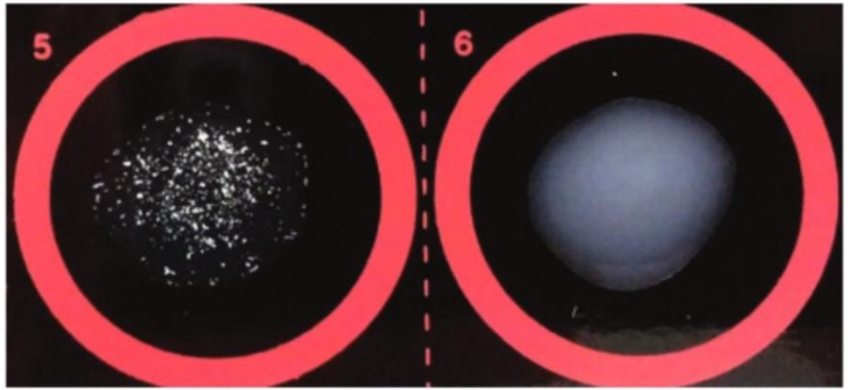
If it is necessary to know whether a serum sample has antibodies directed against a particular red blood cell and you want to be sure that you also detect potential **non- agglutinating antibodies** in the sample, an Indirect Coomb's test is performed.

This test is done by **incubating the red blood cells** with the **serum sample**, washing out any unbound antibodies and then adding **a second anti-immunoglobulin reagent** to cross link the cells.



5- PASSIVE AGGLUTINATION TEST

- To detect antibodies against soluble antigen using agglutination test.
- Soluble Antigen + **inert carrier particles** = Particulate antigen
- Carrier particles - Red cells, **latex**, bentonite → **passive hemagglutination**.
  - **Latex agglutination test :** ASO, CRP, RA factor



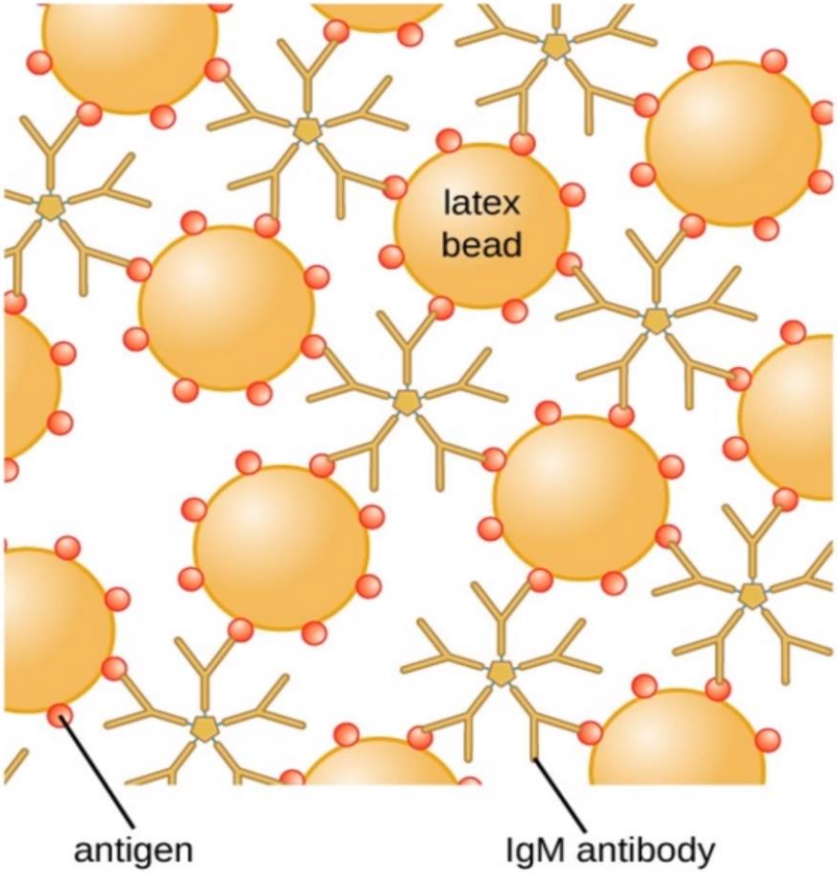
Positive LAT Negative LAT

Passive Agglutination Test

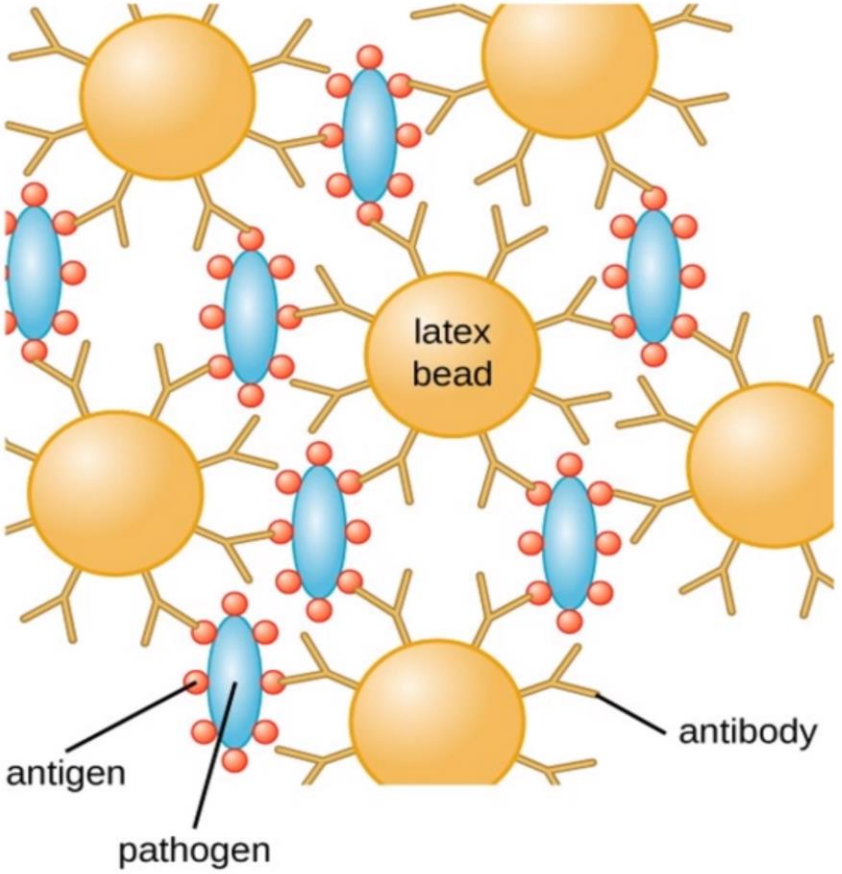
- Bind known **ANTIGENS** to inert particles to detect antibody.
- Reactions easy to read macroscopically.
- Many antigens adsorb onto RBCs spontaneously, **tanned sheep RBCs** frequently used.
- IgG naturally adsorbs onto surface of latex particles.

Reverse Passive Agglutination Test

- Bind known **ANTIBODY** to carrier particle instead of antigen.
- Widest application is in detecting soluble antigens in urine, spinal fluid and serum.
- Antigens present in these fluids will attach to antibodies on particles.



(a) positive agglutination test for antibodies



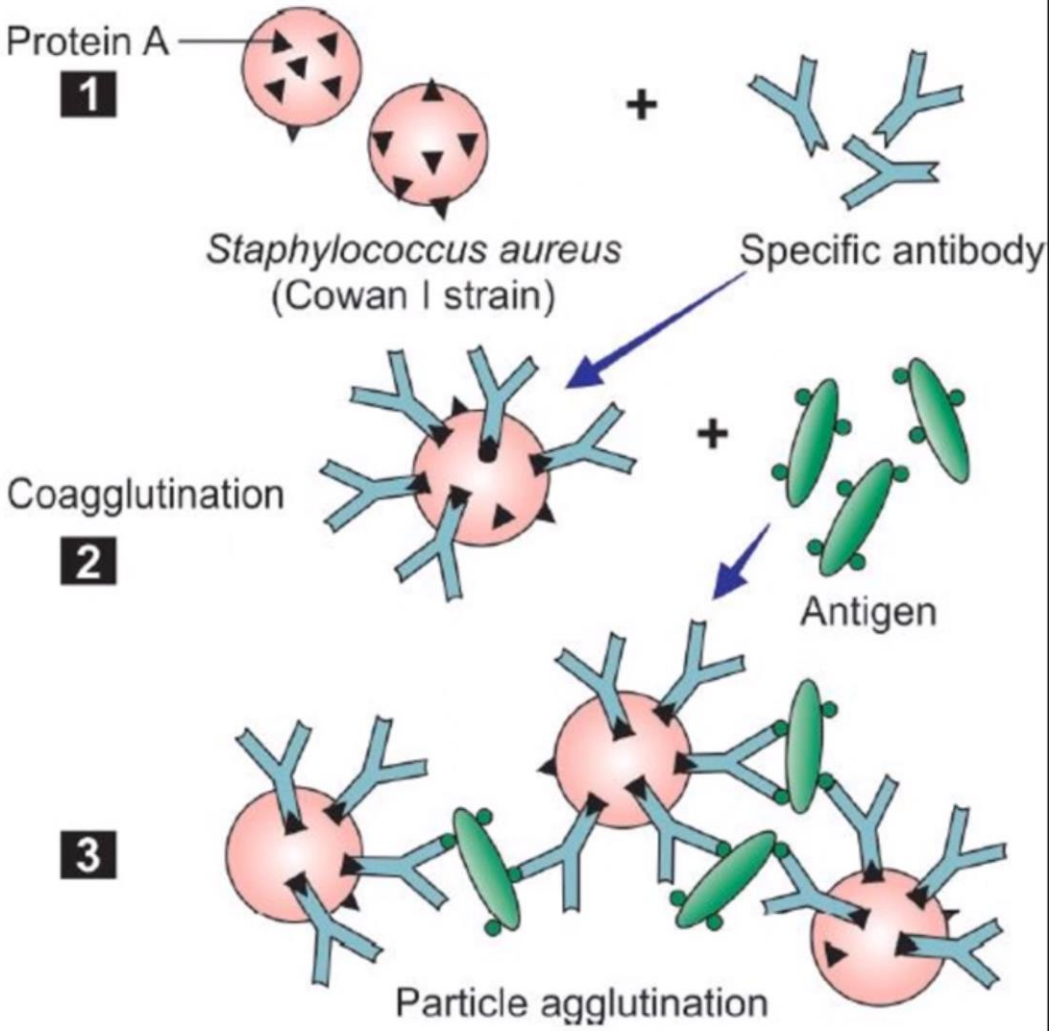
(b) positive agglutination test for antigens

Passive Agglutination Test vs. Reverse Passive Agglutination Test

6- CO-AGGLUTINATION

- Protein A bearing Staphylococci coated with any IgG antiserum will be agglutinate if mixed with corresponding antigen.

e.g. Detection of early *Salmonella* infection

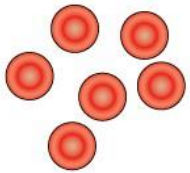
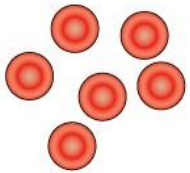
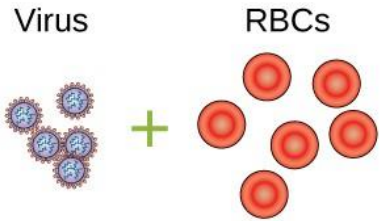
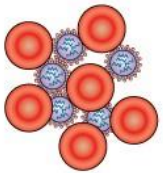
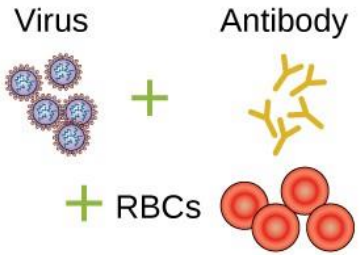
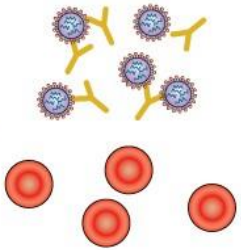


7- Hemagglutination Inhibition (HI)

Based on competition between particulate and soluble antigens for limited antibody combining sites.

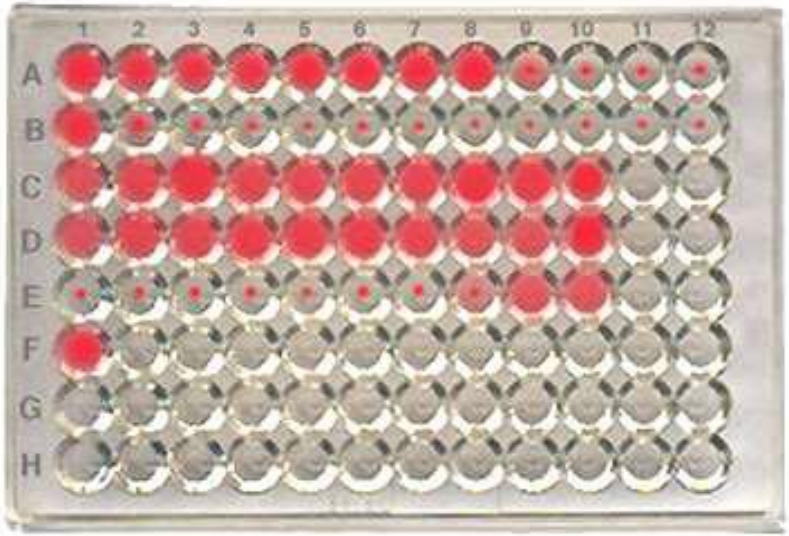
- Patient sample added to reagent antibody specific for antigen being tested, if antigen is present it binds to reagent antibody.
- Reagent particles (latex or RBCs) coated with the **same antigen** are added, if antigen was present in the sample all reagent antibody binds to it so no antibody is present to react with antigens coating the particles

NO agglutination = POSITIVE reaction.

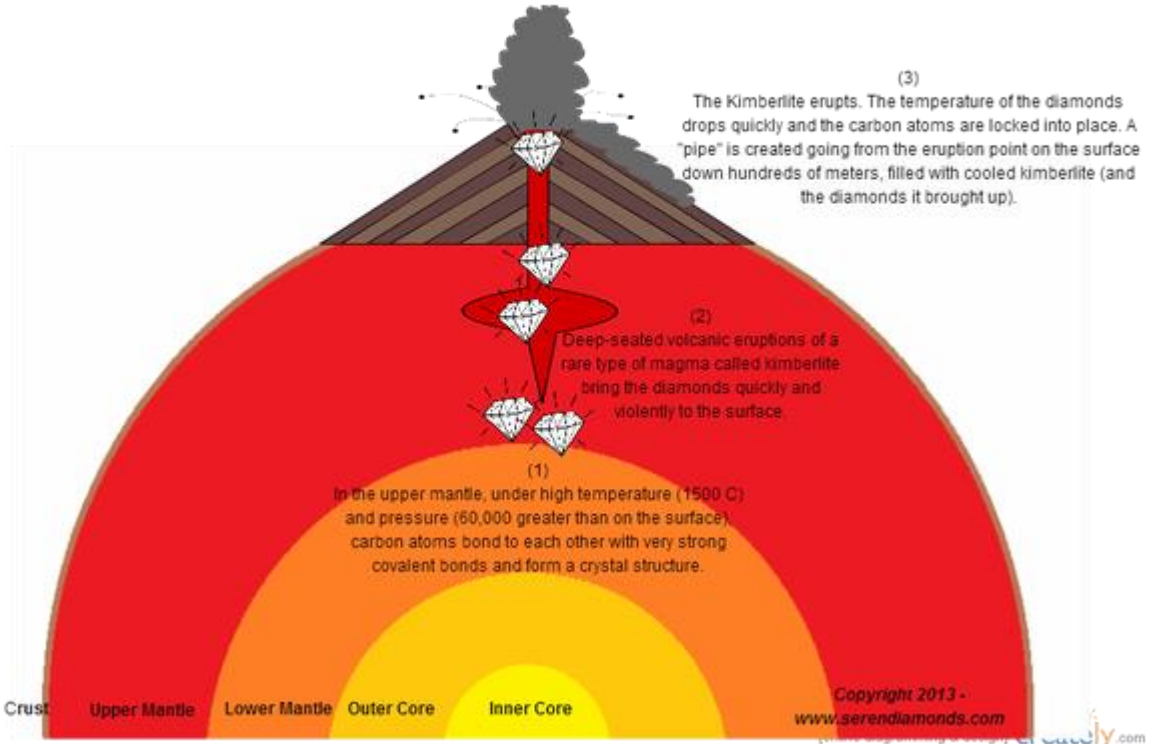
	Components	Interaction	Microtiter Results
A	RBCs 		No reaction 
B	Virus + RBCs 		Hemagglutination 
C	Virus + Antibody + RBCs 		Hemagglutination inhibition 

Let's interpret the results together

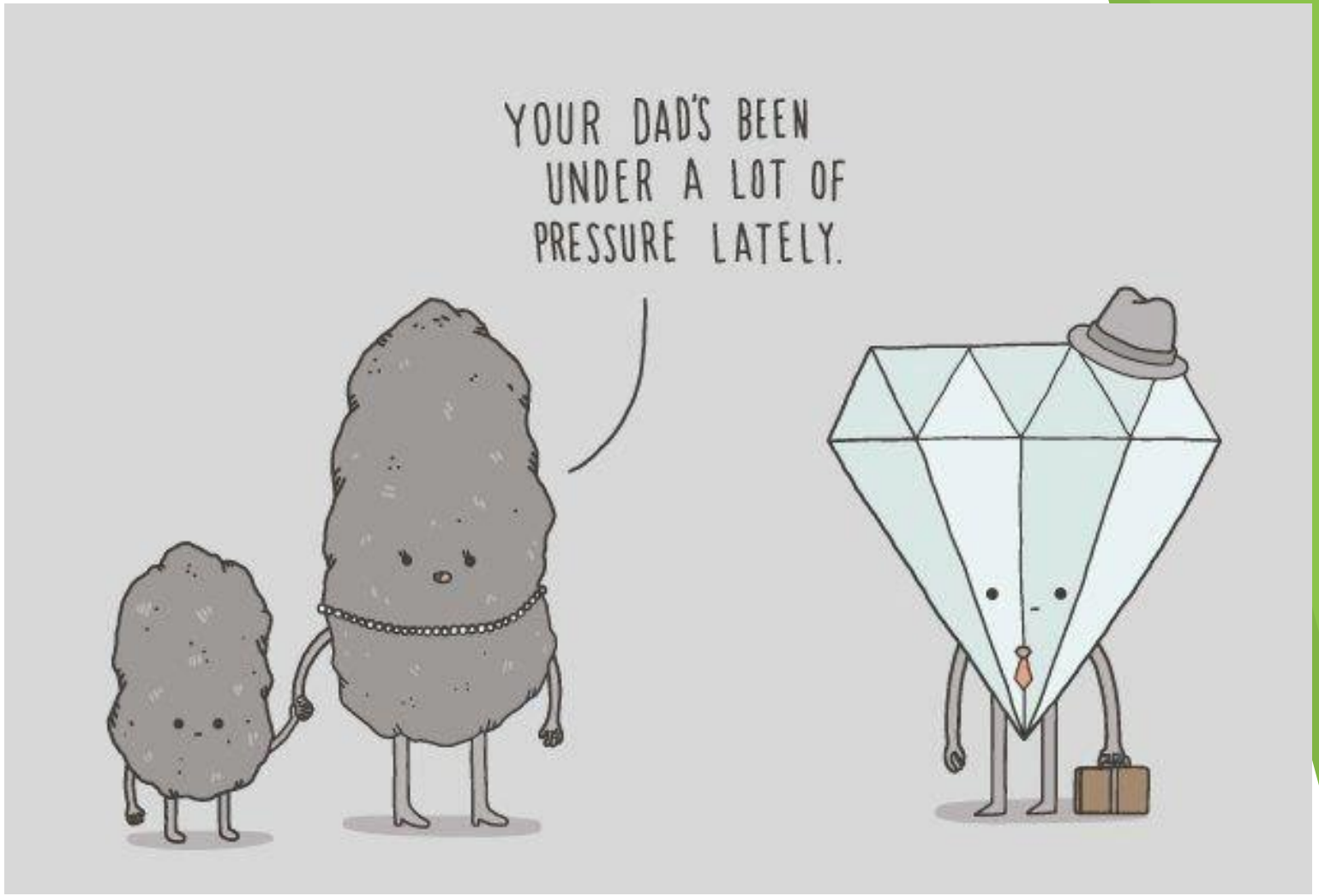
- In wells if agglutination occurs the clumps cover the well.
- No agglutination will allow the RBCs to flow down sides and collect at the bottom.



How Are Diamonds Formed?



“ When life gets hard remind your self,


YOUR DAD'S BEEN UNDER A LOT OF PRESSURE LATELY.

THANKS!

Any questions?

You can find me at third floor office 87

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