Lab-3-
Laboratory Diagnosis
Immunological Tests

Submitted by

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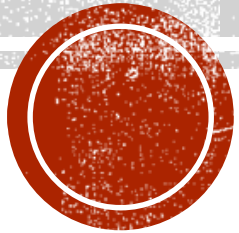


4-Hemagglutination.

Principle of Haemagglutination (HA)Test

Certain viruses haemagglutinate erythrocyte due to the binding of Haemagglutinin – Neuraminidase (HN) Protein of Virus to Receptors on Surface of RBCs .

*Many human viruses have the ability to bind to the surface structures on red blood cells from different species there by causing agglutination.

Haemagglutination Phenomenon is defined as ability of Certain viruses (haemagglutination viruses) to aggregate RBC IN Suspension.is agglutination that involves red blood cells(RBC).

EXAMPLE

1-Blood typing

2-Quantitative of virus dilute in aHaemagglutination Assay

Influenza virus binds to fowls red blood cells.

Reagent and Conditions for the Test Vary by Virus.

The Agglutinate appear as Precipitate of RBC in the well of Plate.

Material of Haemagglutination test(HA)

1-HA Plate (Microtiter plate or Macrotiter Plate)

2-Macropipete & Micropipete

3-Chemical agent such as PBS (Phosphate Buffer Solution) or Normal Saline or EDTA.

4- Biological agent such as Washed RBC (The blood sample was taken from sheep, Guinea pig , chicken , Rabbit and Humen) .

5-Virus: Which taken from Vaccine ampule or infected Chicken.

The aim (Use) of the Haemagglutination (HA) Test:

1-Titeration The virus

2-Measure of HA unit.

3-Diagnosis the Virus which have the ability to agglutinate of RBCs.

Procedure of Haemagglutination (HA)Test

1-Add (0.5ml) of N.S to all wells and add(0.5ml) of the Virus to the first well and Mixed and then transfer by Micropipete to the next well and third well,…etc (Make Serial dilution 2 fold)and finally 0.5ml discard.

2-Add the (washed RBC 1%) to each well.

3- Make the Two Control well by adding blood to each well then put the virus in the one well and normal Saline on the others.

4-Mixed by tapping the plate gently , then allow RBC to settle for about (30 minute). Haemagglutination is Observing by the presence or Absence of tear (net) shape of RBC.

5- The Titration should be read to highest dilution giving complete HA.

6-Method of Wash RBC:- Take (1ml) of blood with EDTA in Tube , if 1ml blood can put (2ml)of D.W(1BLOOD:2 D.W) in Centrifuge can result RBC , Buffy coat, Plasma by pipette can draw the plasma and buffy coat content and repeat the method for 3 times to get RBC Washed.

Result of Haemagglutination (HA)Test

The concentration of Wash RBC(0.5-1%).

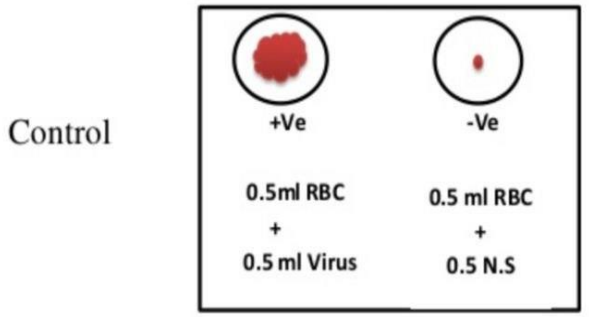
1ml of RBC+99ml of N.S=1%

IF UNIT IS 0.5%

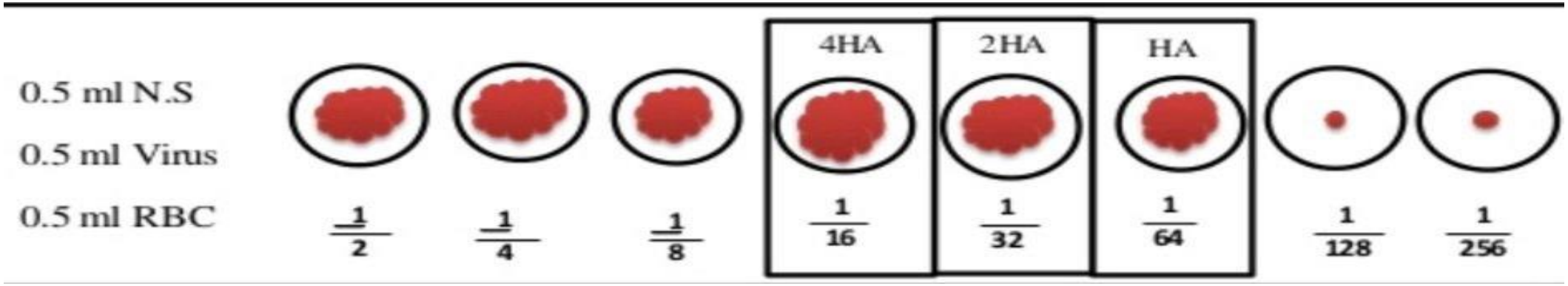
C1V1=C2V2

1%*100ml=V2*0.5%

V2=1*100/0.5=200ml



معيار الفايروس= مقلوب أعلى تخيف اعطى نتيجة موجبة × 2



4-Agglutination Inhibition .

.Principle

* Agglutination inhibition - based on competition between particulate and soluble antigens for limited antibody .

* combining sites, and a lack of agglutination is an indicator of a positive reaction.

Example

- human chorionic gonadotropin (HCG) and antibody to HCG.

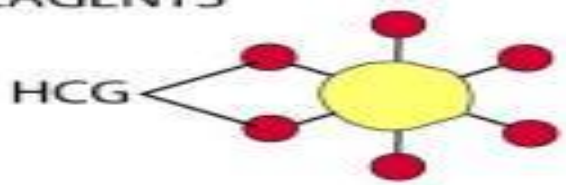
4-Agglutination Inhibition .

.Example:

- *The classic example of agglutination inhibition is the early types of home pregnancy test kits included latex particles coated with human chorionic gonadotropin (HCG) and antibody to HCG

* The addition of urine from a pregnant woman, which contained HCG, inhibited agglutination of the latex particles when the anti-HCG antibody was added; thus the absence of agglutination indicated pregnancy.

KIT REAGENTS



and

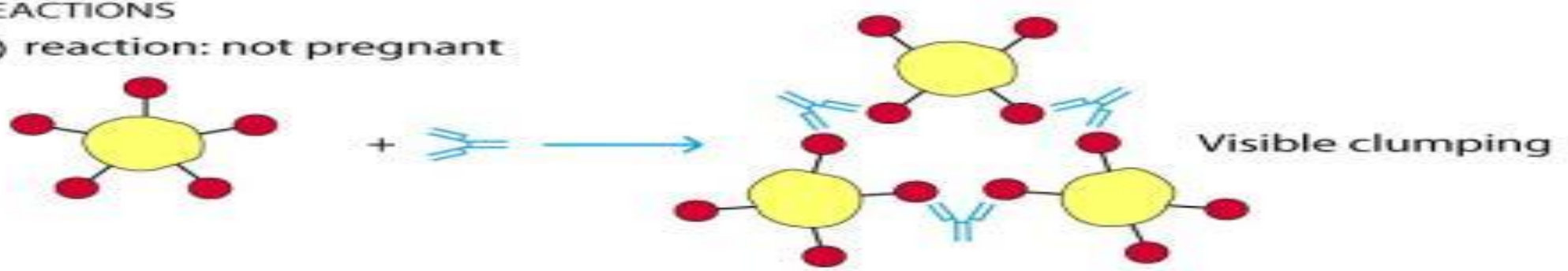
Hapten carrier–conjugate Anti–HCG antibody

TEST PROCEDURE

Urine + Anti–HCG → (Incubate) + HCG carrier conjugate → Observe for visible clumping

POSSIBLE REACTIONS

⊖ reaction: not pregnant



⊕ reaction: pregnant

4-Hemagglutination Inhibition .

.Principle

***Antibodies to the Virus in the Patient Serum bind to the Virus; Blocks binding Sites on the Viral Surface.**

***Prevents the virus from agglutination the red blood cells.**

Example

-Detecting Antibodies to influenza and Dengue viruses.

Haemagglutination Inhibition (HAI)Test

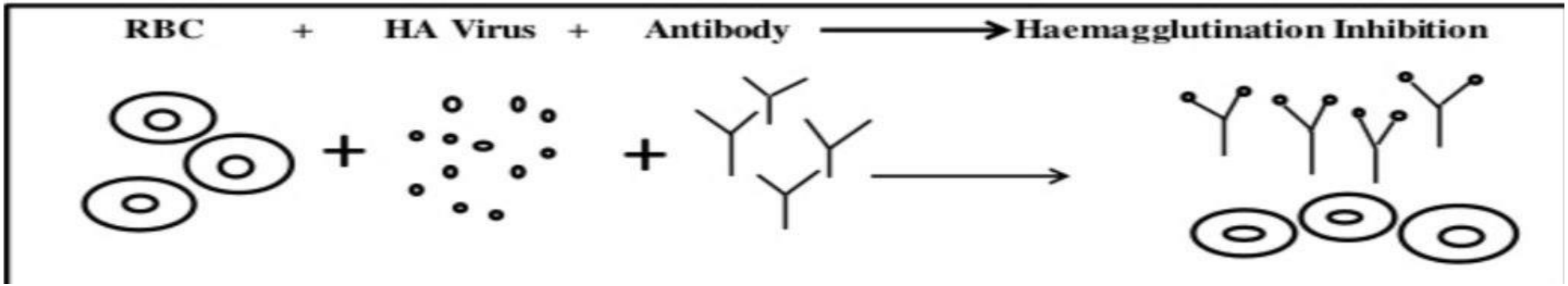
Principle of (HAI) TEST

Specific attachment of Antibody to Antigen Sites (on HA Molecule of Virus) interfere with binding between the virus (Haemagglutinate) and receptors on the RBC.

Definition: It is a Serologic test depending upon antigen – antibody reaction in which inhibition of Haemagglutination occurs due to masking of virus receptors by specific Antibodies.

used for diagnosis of infection produced by (Orthomyxovirus, Paramyxoviruses and the arboviruses – togaviruses (Rubella), Flaviviruses and bunyaviruses.

*Antibody can be detected in patients by inhibition of virus



Viral Neutralization

Definition: It used Frequently in diagnosis of Viral Infection Such as influenza, Mumps and Measles.

If the Serum of Patient Contains Antibodies against Certain Viruses that have the property of agglutinating the red blood cell , these antibodies react with the viruses and inhibit the agglutination of the RBCs

Procedure of HAI Test

1-Add (0.5ml) of N.S to each well U –Bottomed of Macro titer plate.

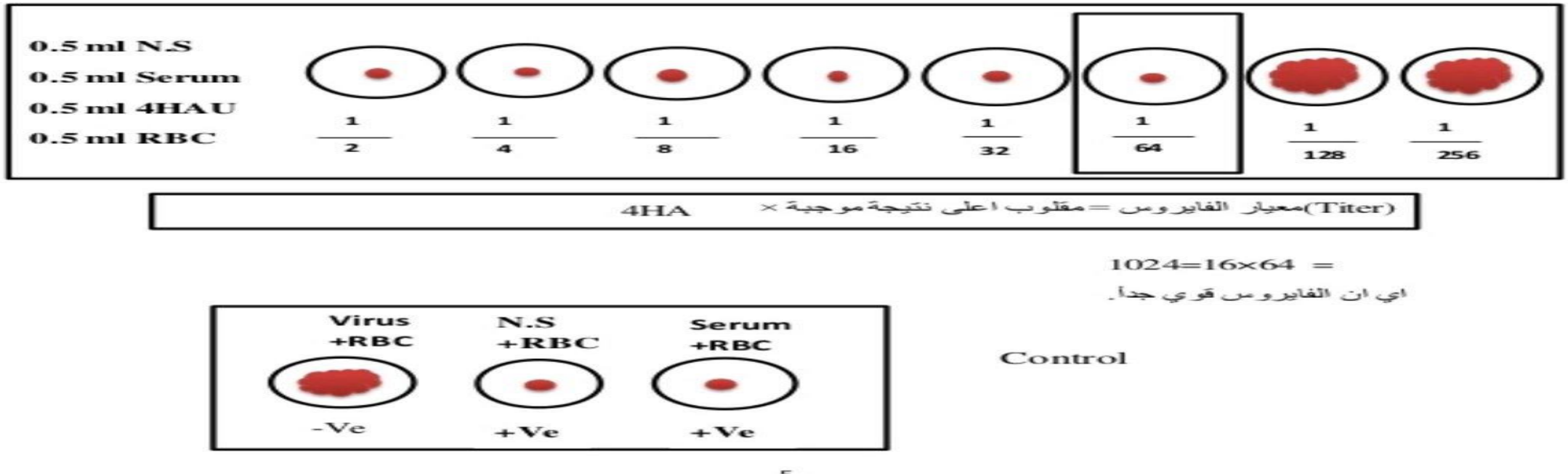
2-Add (0.5ml) of Serum (Abs) in the first well of plate then Make Serial dilution (Add to first well and Mixed and then transfer to next well and third well …etc) and finally discard 0.5ml.

3-Add(0.5ml) of 4HAU to each well.

4-Leave for(30minute) at room temperature.

5-Add (0.5ml) of washed RBCs to each well.

6-Mixed gently then allow RBC to settle for about (30minute).



معيار الفايروس (Titer) = مقلوب اعلى نتيجة موجبة × 4HA

1024=16×64 =

اي ان الفايروس قوي جداً.

The aim (Use) of the Haemagglutination Inhobition (HAI) Test:

1- Titration of Antibody

2-Evaluation of Vaccine

3-Typing of Haemagglutination Virus (using antisera) e.g influenza

Hemagglutination and Hemagglutination Inhibition

Advantages

*Highly Specific

*Can be used as gold Standard

Lmitations

*Technically demanding

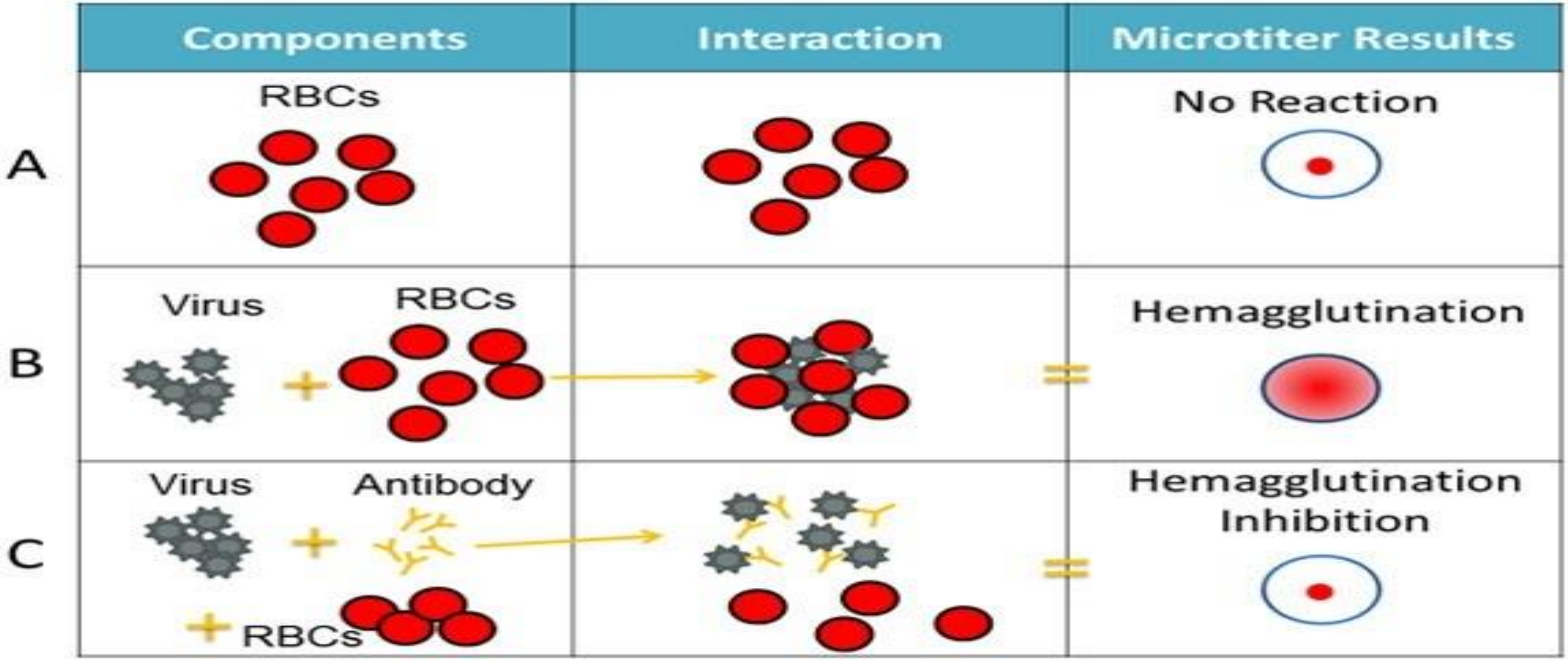
*Time consuming

*Cannot distinguish IgG from IgM

Time taken

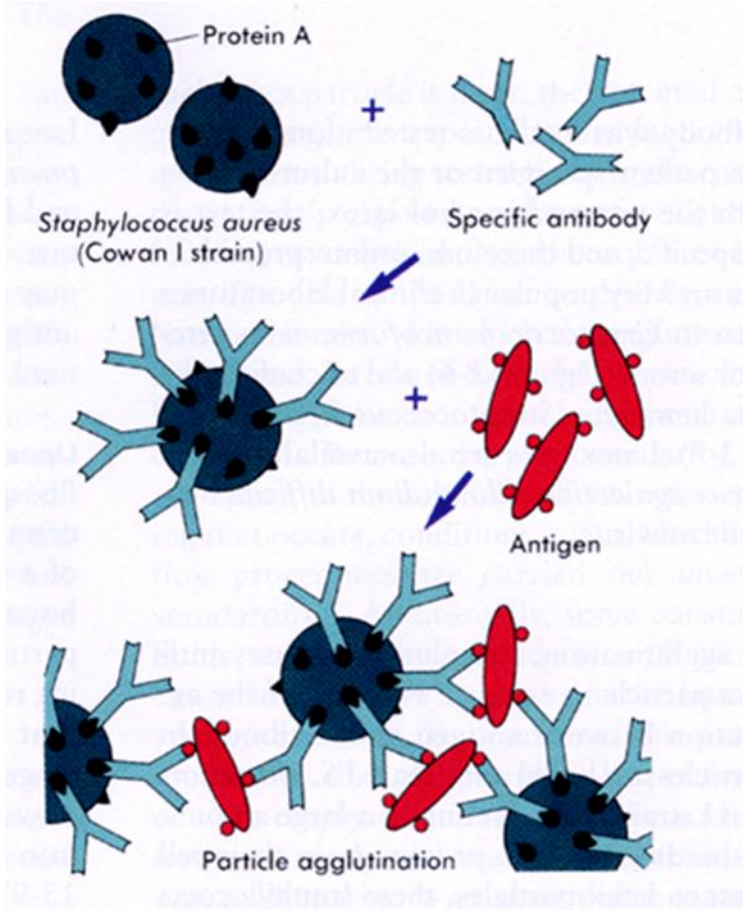
*1 day

Hemagglutination and Hemagglutination Inhibition

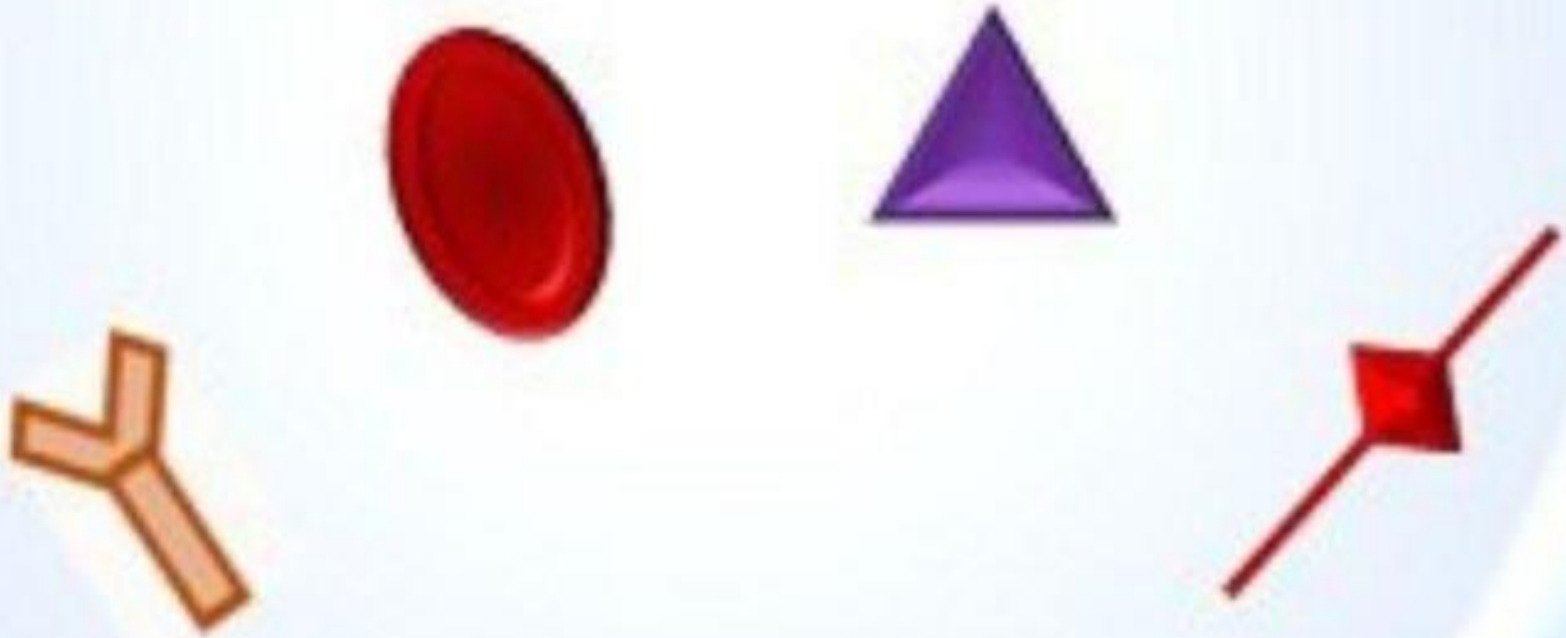


Coagglutination/Conglutination

- Coagglutination/Conglutination - name given to systems using bacteria as inert particles to which antibody is attached (S. aureus).
- The Fc region of antibody attaches to protein A of staphylococcal cell leaving the Fab region to combine with the antigen
- Killed staphylococcal cells coated with antibody can be used to identify bacteria and detect soluble extracellular bacterial antigens in specimens and body fluids.



COMPLEMENT FIXATION TEST



Complement Fixation test

Test System:

***Antigen: it may be Soluble or Particulate.**

***Antibody: Humen Serum (May or not contain Antibody towards Specific Antigen).**

***Complement: It is pooled serum obtained from 4 to 5 guinea pigs . It should be fresh or Specially Preserved as the complement activity in heat Labile(stored at -30 in small fraction)**

Indicator System:

***Erythrocytes: Sheep RBC**

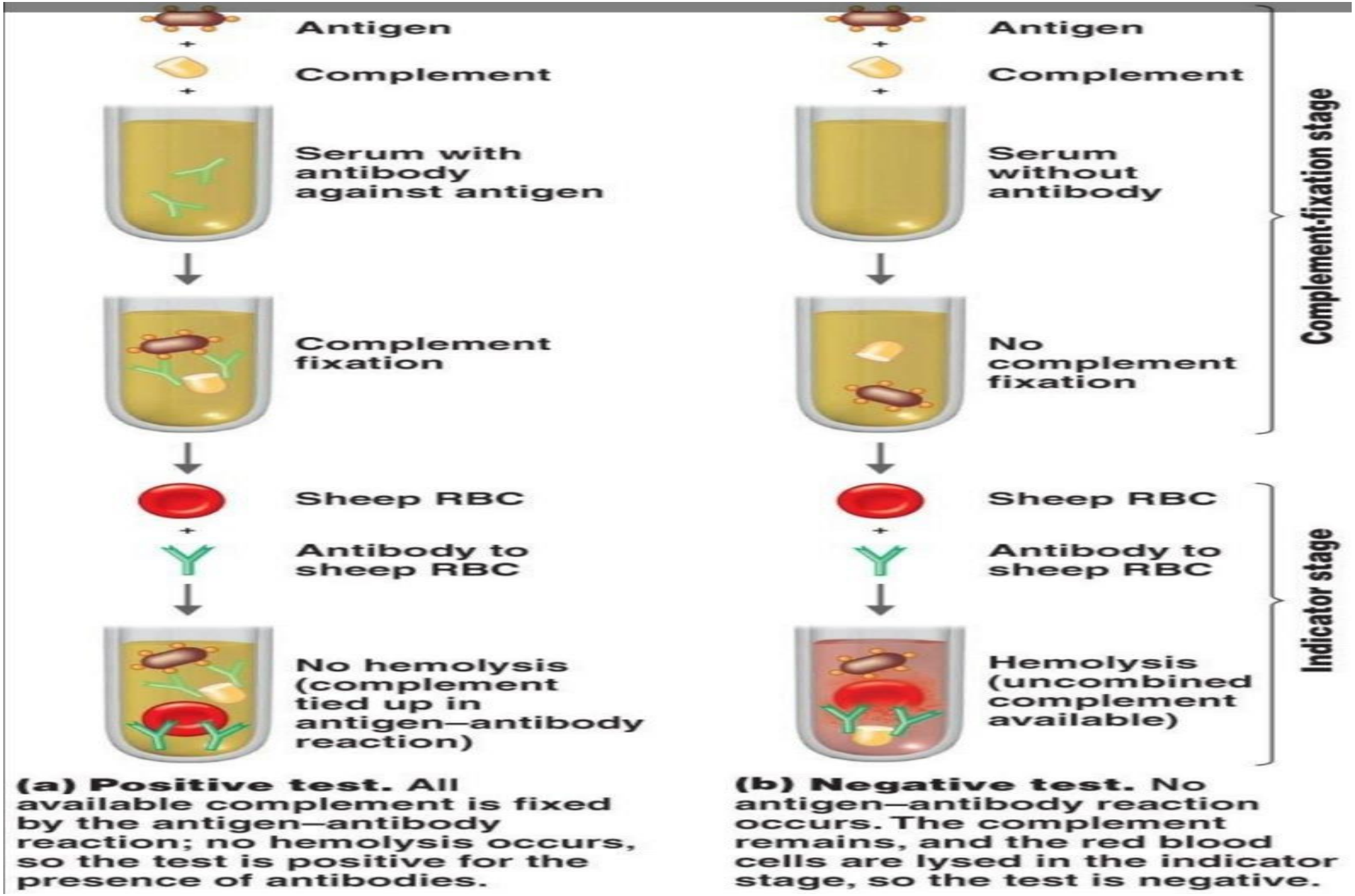
***Amboceptor (Hemolysin): Rabbit antibody to sheep red cells preparing by inoculating sheep erythrocytes into rabbit under standard immunization porotocol.**

Principle :

-Virus antibody is detected by fixation of added complement when the Ab combines with virus Ag.

-Fixation rendered visible by Latter addition of Sheep erythrocytes Sensitized by addition of anti- erythrocyte antibody.

-If Virus antibody present , complement is fixed and the sheep red Cells do not haemolysed. If no virus Ab present , the complement lyses the Sensitized erythrocytes.



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Complement Fixation test

Negative Test

- **Step 1:**

Antigen + Antibody absent + Complement —(At 37°C, 1 Hour)→ Complement not fixed

- **Step 2:**

Free Complement + Haemolytic system —(At 37°C, 1 Hour)→ Haemolysis (Test Negative)

Positive Test

- **Step 1:**

Antigen + Antibody + Complement (from serum) —(At 37°C, 1 Hour)→ Complement gets fixed

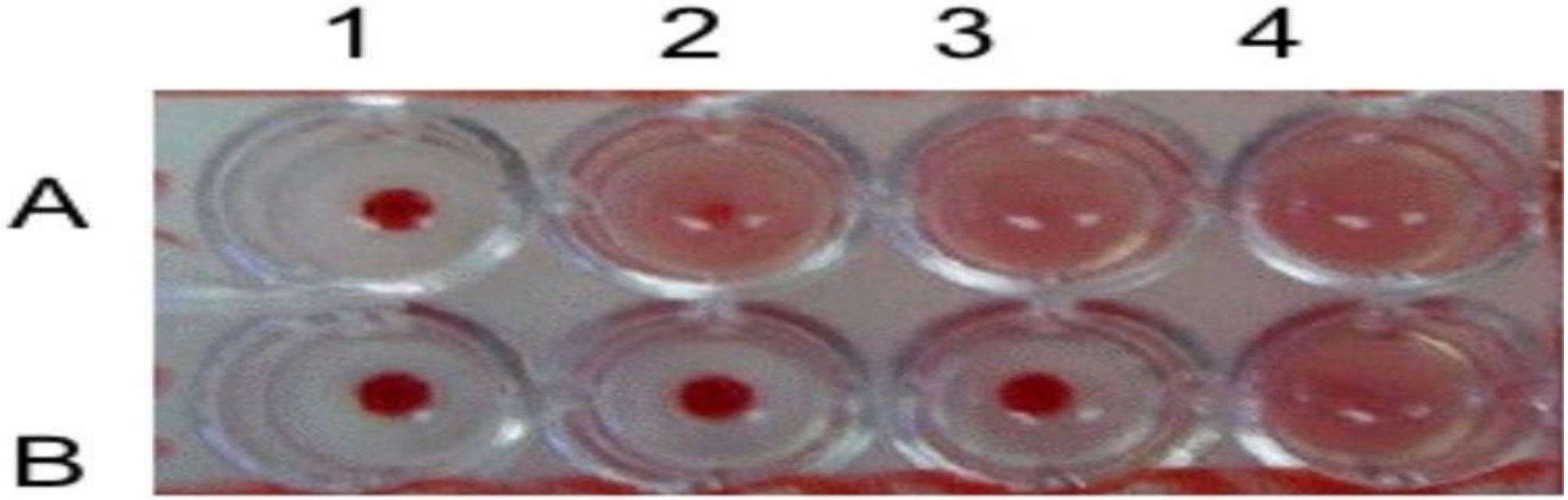
- **Step 2:**

Fixed Complement complex + Haemolytic system —(At 37°C, 1 Hour)→ No Haemolysis (Test Positive)

Result and Interpretations of CFT

Positive test: the available complement is fixed by Ag-Ab complex and no hemolysis of sheep RBCs occurs , So the test is positive for presence of Antibodies. Show in A1, B1,B2,B3)

Negative test: No Ag-Ab reaction occurs and the complement is free . This free Complement binds to the complex of sheep RBC and its Antibody to cause hemolysis , causing the development of pink color. Show haemolysis in (A3.A4 and B4).



Complement Fixation test

Advantages of CFT

1- Ability to screen against a large number of viral and bacterial infections in same time.

2- Economical.

Disadvantages of CFT

1-Not Sensitive – can not be used for immunity screening

2-Time consuming and labor intensive.

3-Often non- Specific.


THANK YOU