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Course Title : Clinical Microbiology

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Unit-IV

Serological Diagnosis of Virus infections

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SEROLOGICAL DIAGNOSIS OF VIRUS INFECTIONS

INTRODUCTION

- Serological methods are diagnostic methods that are used to identify antibodies and antigens in patients sample which is serum and plasma.
- A serological diagnosis is prepared by detecting the rising titres of antibody or antigen
- By serological methods most of the common viral infections are diagonized.

SEROLOGICAL REACTIONS

Ag-Ab reactions used for the detection of unknown Ag or Ab, in vitro In virology

- ✓ **Viral (direct) diagnosis: - detection of viral antigens**
- ✓ **Serological (indirect) diagnosis: - detection of specific antiviral antibodies**

it is important for diagnosis of viral infection

- **Proper**
- **Sample collection-proper site,proper time, quantity (saliva),swabs**
- **Transportation-VTM -protein, antibiotics,ph indicator,not required for sterile body fluid or CSF , should not be diluted.**
- **Preservation->72 hours, avoid repeated freezing& thawing.**
- **Test- HIV in infant**
- **Method-Ag,antibody detection,viral culture**
- **Kits-Higher sensitivity for screeningof HIV .**

VIRAL TRANSPORT MEDIAM

- Viral transport medium (VTM) is a solution used to preserve virus specimens after collection so that they can be transported and analysed in a laboratory at a later time.

- Chemical components may include saline solution, phosphate-buffered saline (PBS), or fetal bovine serum (FBS).

- VTM must be sterile to avoid introducing contamination to the specimen.

GENERAL METHODS OF DIAGNOSIS SEROLOGY

Recommended specimens for viral diagnosis

S.N.	Source	Specimens	Common Viruses detectable
1.	Respiratory tract	Nasal washing, throat swab, sputum, nasal swab	Adenovirus, Influenza, Enterovirus(picorna virus), Rhinovirus, Paramyxovirus, Rubella virus, Herpes virus, etc.
2.	GI tract	Stool, Rectal swab	Reovirus, Rotavirus, Adenovirus, Norwalk virus, Calicivirus, etc.
3	Eye	Swabs	Herpes virus, etc.
4	Maculopapular rash	Throat swab, Swabs from infected site	Adenovirus, Enterovirus (picornavirus), Rubella virus, Measles, etc.
5.	Vesicular rash	Vesicle fluid, Scrapping or swab, Feces (for Enterovirus)	Coxsackie virus, Echovirus, HSV, VZV, etc.
6.	CNS (Aseptic meningitis/encephalitis)	Tissue, saliva, brain biopsy CSF, Brain biopsy	• Enterovirus, Arbovirus(e.g. togavirus, bunya virus), Rabies • HSV,CMV, Mumps, Measles, etc.
7.	Urinary Tract	Urine	Adenovirus, CMV, etc.
8	Blood	Whole blood, Serum, plasma	HIV, Human T-lymphocyte Virus, Hepatitis virus(B,C and D), etc.

METHODS OF DIAGNOSIS

SEROLOGY

Classical techniques

1.complement fixation test(CFT)
2.Haemagglutination inhibition test
3.Immunofluorescence techniques (IF)
4.Neutralization test
5.Counter Immunoelectrophoresis

Advanced techniques

1Radioimmumoassay(RIA)
2.ELISA
3.Western blot(WB)

1.ELISA

- ✓ **ENZYME LINKED IMMUNOSORBRNT ASSAY**

- ✓ **ELISA (which stands for enzyme-linked immunosorbent assay) is a technique to detect the presence of antigens in biological samples.**

- ✓ **An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions.**

- ✓ **ELISA is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.**

- ✓ **Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.**

PRINCIPLES OF ELISA

- ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity.
- ELISA can provide a useful measurement of antigen-antibody concentration.

TYPES OF ELISA

ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely:

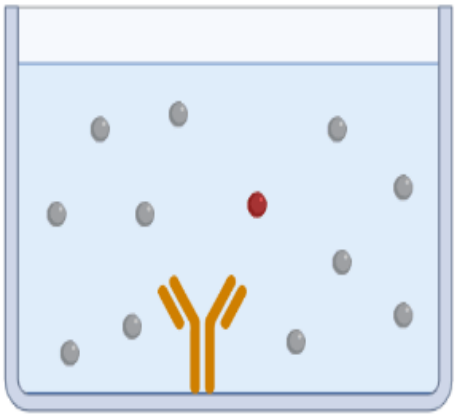
- *Indirect ELISA – Antigen is coated to the microtiter well*
- *Sandwich ELISA – Antibody is coated on titer microtiter well*
- *Competitive ELISA – Microtiter well which is antigen-coated is filled with the antigen -antibody mixture.*

PROCEDURE

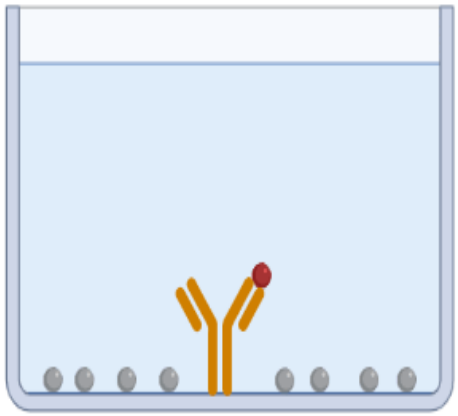
- An antibody is attached to a polystyrene plate which is a solid surface and is attracted or has an affinity towards bacteria, other antibodies and hormones.
- A microtiter coated with antigen is filled with this antigen-antibody mixture after which free antibodies are removed by washing.
- A second antibody specific to primary antibody is added which is usually conjugated with an enzyme.
- Free enzyme-linked secondary antibodies are removed by washing the plate.
- Finally, the substrate is added. The substrate is converted by the enzyme to form a coloured product, which can be measured by spectrophotometry.

ELISA can be used to detect some of these conditions:

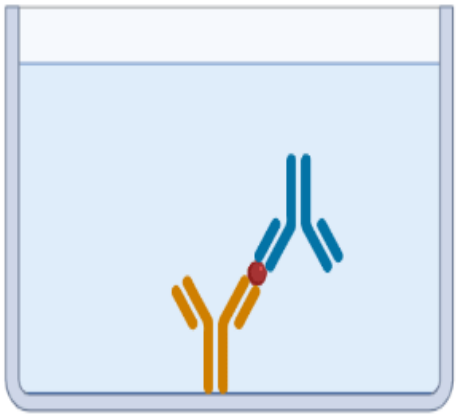
- *Ebola*
- *Pernicious anaemia*
- *AIDS*
- *Rotavirus*
- *Lyme disease*
- *Syphilis*
- *Toxoplasmosis*
- *Zika virus*
- *Carcinoma of the epithelial cells*



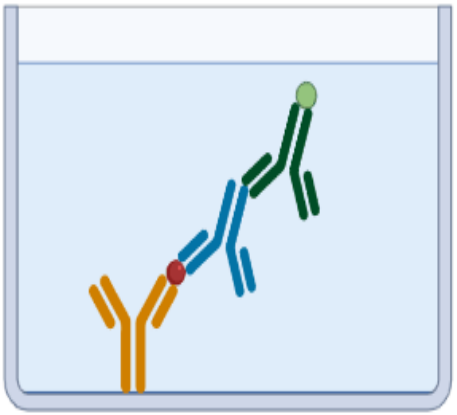
1 Wells are pre-coated with capture antibody and sample is added



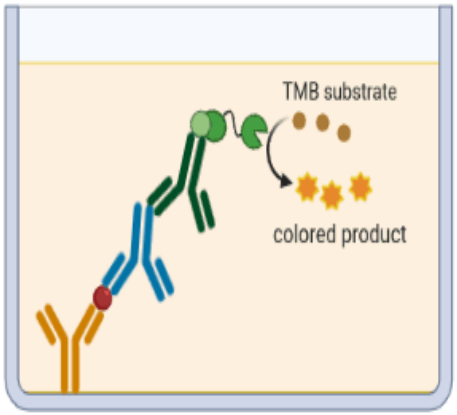
2 Capture antibody binds to antigen with high specificity



3 Primary antibody binds the immobilized antigen



4 Enzyme labeled antibody binds to Fc region of detection antibody



5 Substrate is catalyzed by the enzyme and gives color

ADVANTAGES

- ✓ Results fetched from ELISA gives an accurate diagnosis of a particular disease since two antibodies are used.
- ✓ Can be carried out for complex samples as the antigen is not required to get purified to detect.
- ✓ It is highly responsive since direct and indirect analysis methods can be carried out.
- ✓ It is a rapid test, yields results quickly.
- ✓ Possible detection for ELISA ranges from the quantitative, semi-quantitative, standard curve, qualitative, calibration curve models etc.
- ✓ Easier to perform and uncomplicated process as compared to other assays which require the presence of radioactive materials.

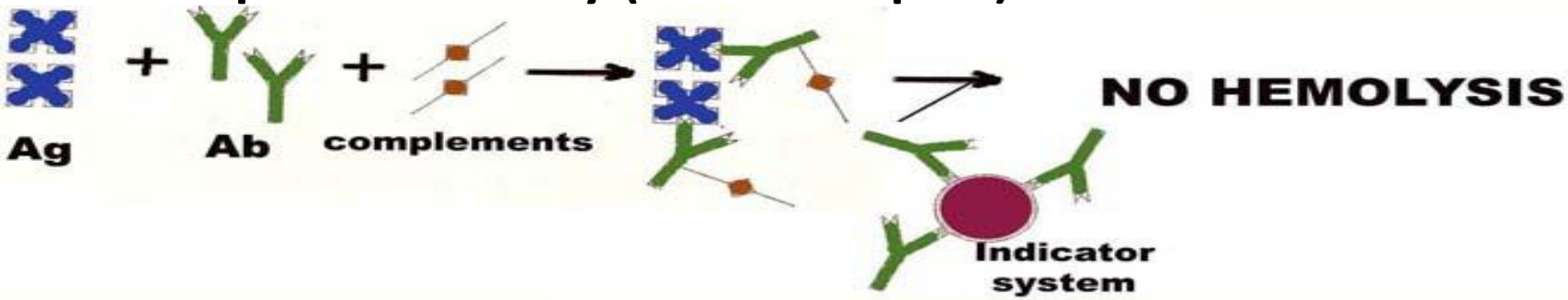
2.COMPLEMENT FIXATION TEST

- Complement fixation test is used to detect and quantify antibody in serum that does not form visible precipitate or agglutinate when reacted with antigen until complement is used.
- Complement is a heat labile globular protein present in normal serum.
- Whole complement system is composed of 9 protein components ie. C1, C2,C3….C9.

PRINCIPLES

- ✓ Complement can only bind Ag-Ab complexes. When complement takes part in antigen-antibody reactions it is bound or fixed to the Ag-Ab complexes.
- ✓ When these complexes are on bacteria, RBCs or other cells, the complement brings about the lysis of these cells. Complement cannot bind free antibody.

- ✓ Antigen-antibody complex fixes the complement. But the fixation of complement with Ag-Ab complex do not have any visible effect like agglutination and precipitation. So it is necessary to use indicators system.
- ✓ The indicator system consists of sheep RBC coated with anti-sheep RBC antibody (Amboreceptor).



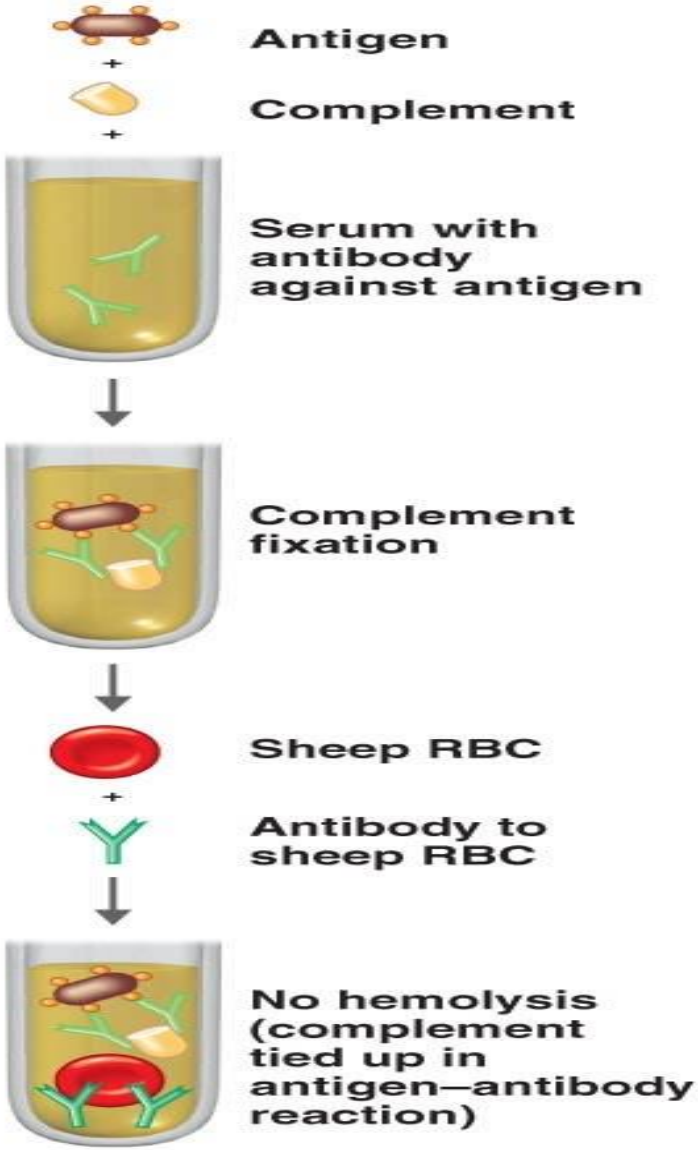
PROCEDURE

- ■ A known antigen (cardiolipin/Viral Ag/sheep RBC) is mixed with inactivated patient's serum
- ■ Add a measured amount of complement (Guineapig serum) in the test system

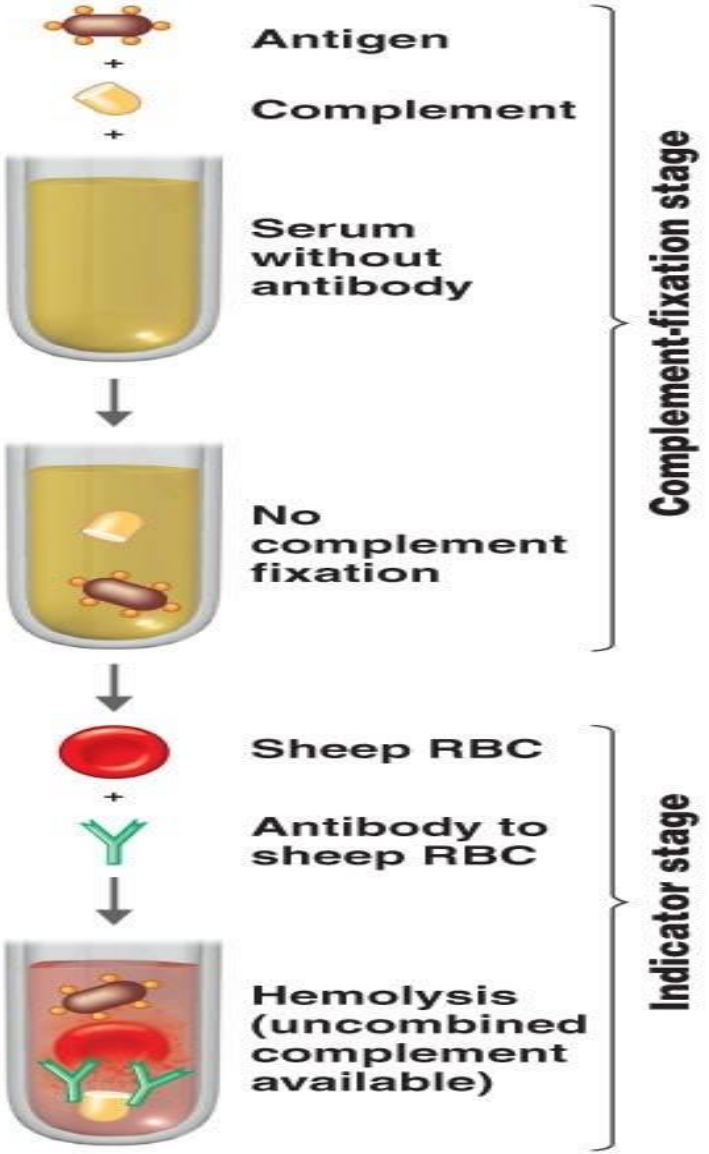
- The test system is incubated at 37°C for about 1 hour.
- After 1 hour an indicator system (sensitized RBC) is added to the test system and again incubated at 37°C for 30 minutes
- If Ag and Ab matches, they form Ag-Ab complex and utilizes complement.
- Observe the result

OBSERVATIONS

- If hemolysis is observed: it indicates the absence of specific antibody in patient serum, so that complement has not been used which lysed the sensitized RBC giving hemolysis.
- If no hemolysis is observed: it indicates that the patient serum contains antibody which reacts with Ag to form Ag-Ab complex and then fix complement .so that no complement is available to hemolyse sensitized RBC.



(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.

RESULT

Positive CFT: if no hemolysis is observed, it indicates positive complement fixation test. Antigen- antibody reaction and complement fixation occurs, so NO free complement is available to lyse the RBC.

Negative CFT: if hemolysis of RBC observed, it indicates negative complement test. NO complement fixation occurs, so the complement remain free and it hemolyse the RBC.

APPLICATION

- ✓ CFT is used to detect and quantify antibody that does not agglutinate or precipitate with its antigen.
- ✓ CFT can detect antibody at level less than 1 microgram per milliliter.
- ✓ CFT is also used to detect antigen
- ✓ CFT is Economical
- ✓ CFT can be used to screen large numbers of viral or bacterial infection.

3.HAEMAGGLUTINATION INHIBITION TEST

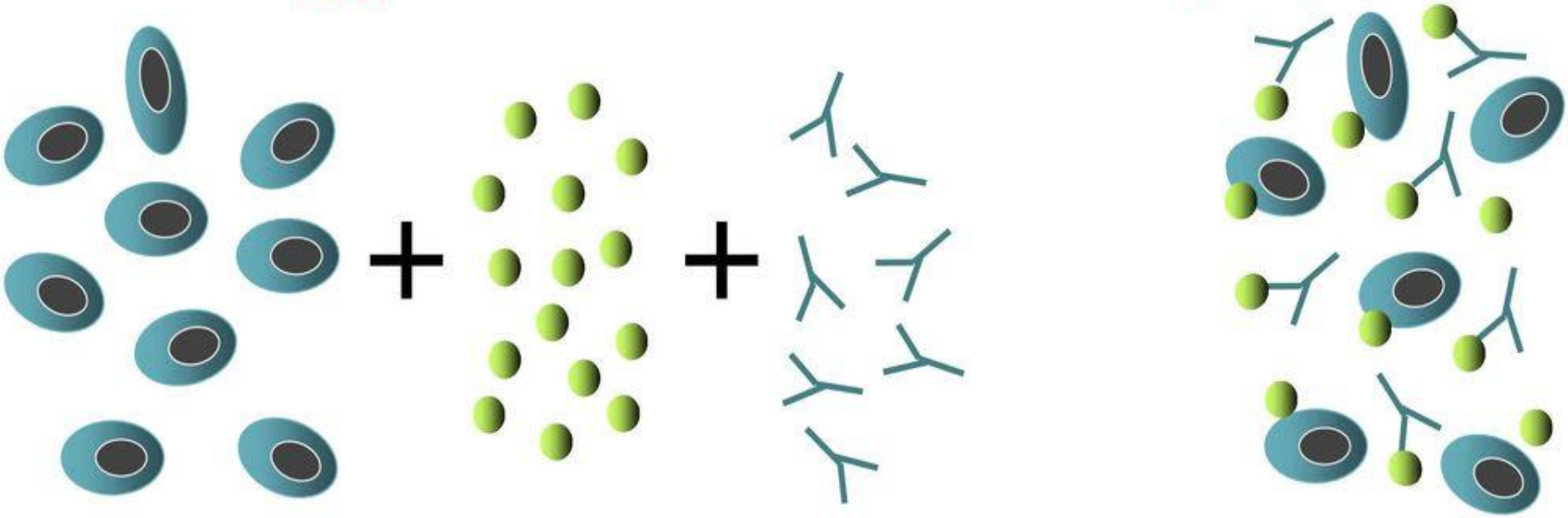
- it a serological test, depending upon antigen,antibody reaction in which inhibition of haemagglutination happens due to masking of virus receptor by the specific antibody.

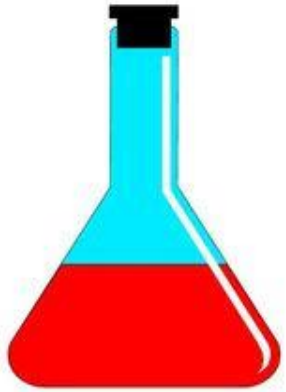
- It is widely used to diagnosis influenza ,measles,mumps and other viral infection.

PRINCIPAL

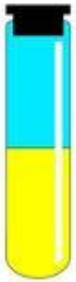
Specific attachment of antibody to antigen sites(on HA molecule of virus) interfere with binding between the virus (haemagglutinate) and receptor on the RBC

Haemagglutination-Inhibition (HI) Test

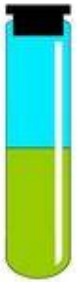




RBC Suspension



HA Virus



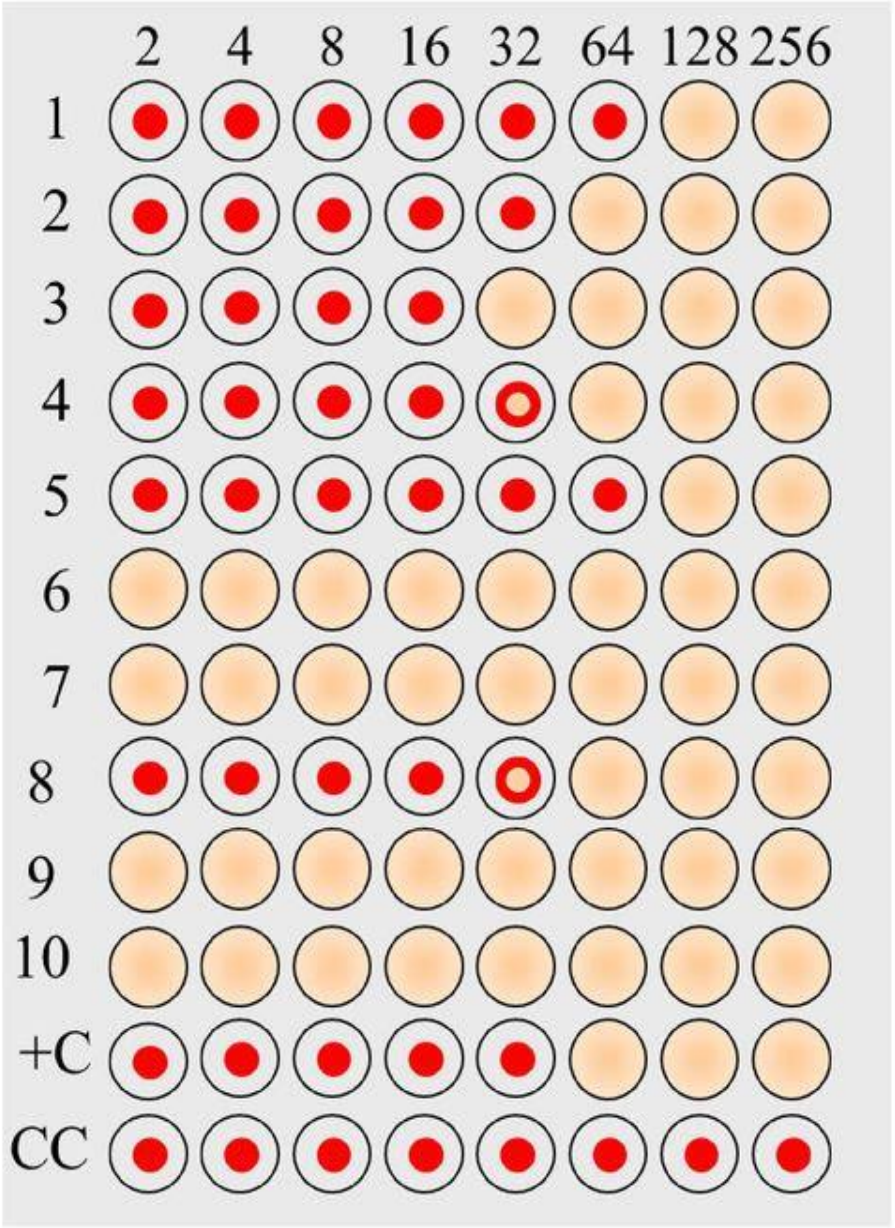
Antibody

Settling Pattern

Procedure

- Label plates (8 or 12 wells/row)
- Add 50 µl PBS to all wells
- Mix & dilute (50 µl) serum through last well
- Add 50 µl virus preparation to each well
- Incubate 30 minutes at room temperature
- Add 50 µl 0.5% washed RBCs.
- Read at 20 – 30 minutes

results



Interpretation

- The highest dilution of serum that prevents haemagglutination is called the HI titer of the serum.
- If the serum contains no antibodies that react with the new strain, then haemagglutination will be observed in all wells.
- Likewise, if antibodies to the virus are present, haemagglutination will not be observed until the antibodies are sufficiently diluted.

REFERENCES

- Pelezar A Jr M .J . Chan and K reig M.R (2007). Microbiology MC graw hill. Inc Newyork.
- Ananthanarayan. R and Ck Jayaram Panikar (1994).Text book of Microbiology.

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