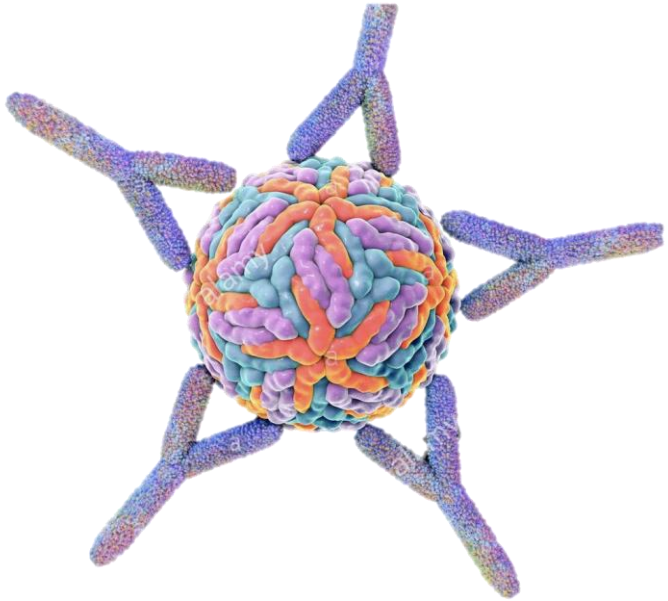
Neutralization



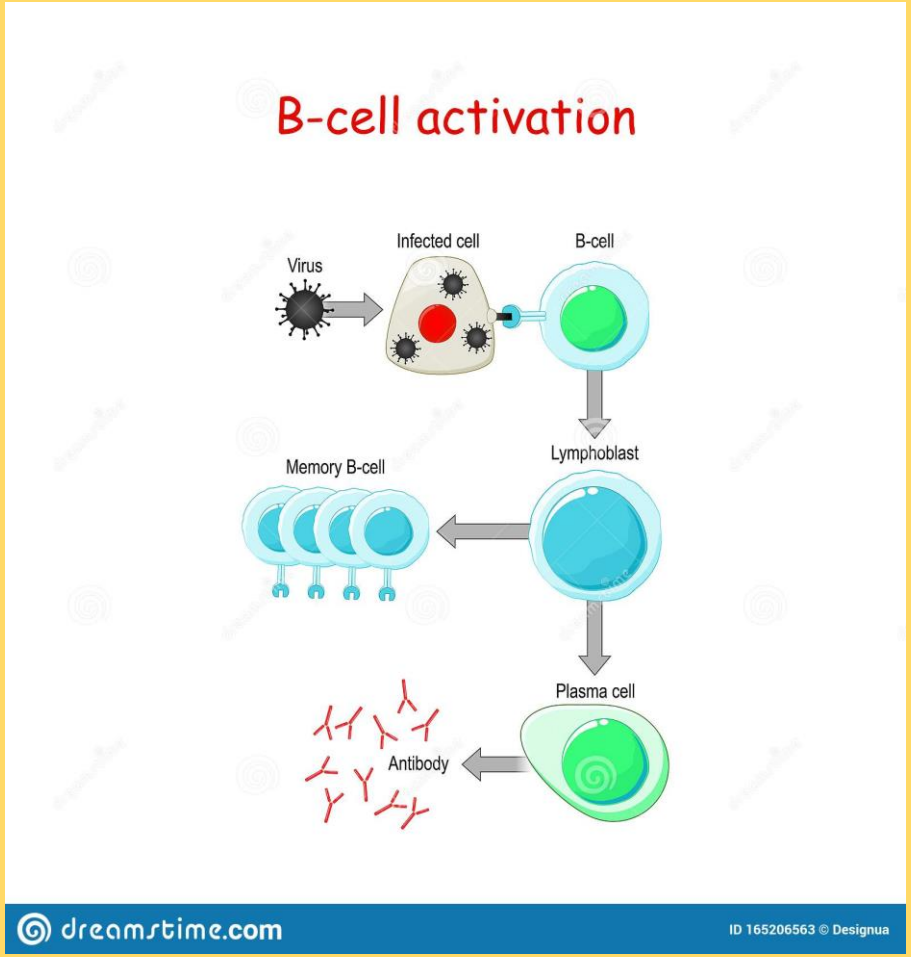
How we diagnose a Virus Infection

Direct	Virus isolation, Antijen detection (EM) Genome detection (eg PCR)	VIROLOGICAL
indirect	Detection of Antibody	SEROLOGICAL

➢ Previously on Virology I;

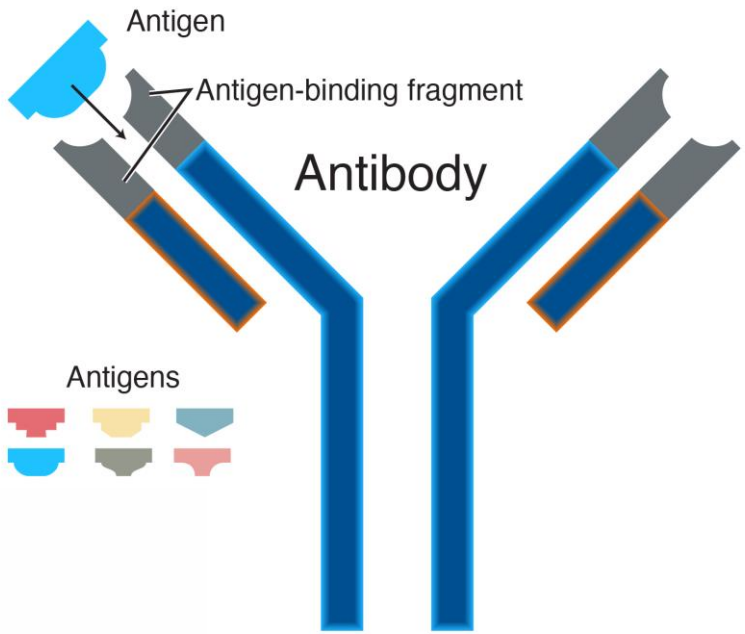
Humoral immune response

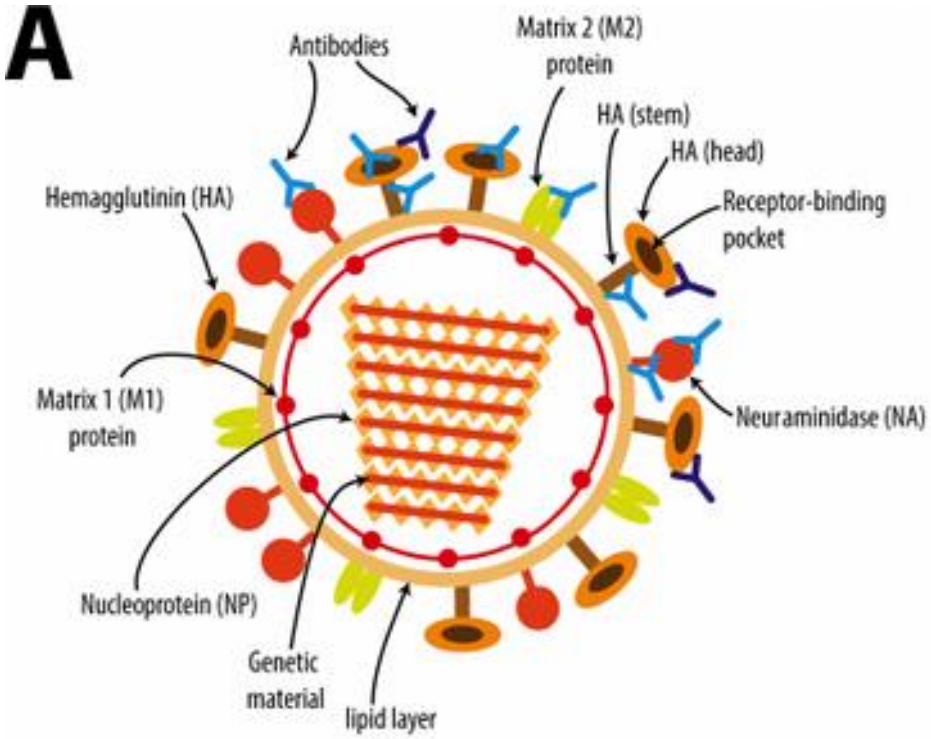
1. B lymphocytes recognize specific antigens
   - proliferate and differentiate into antibody-secreting plasma cells
2. Antibodies bind to specific antigens on microbes; destroy microbes via specific mechanisms
3. Some B lymphocytes evolve into the resting state - memory cells



- Antibody:

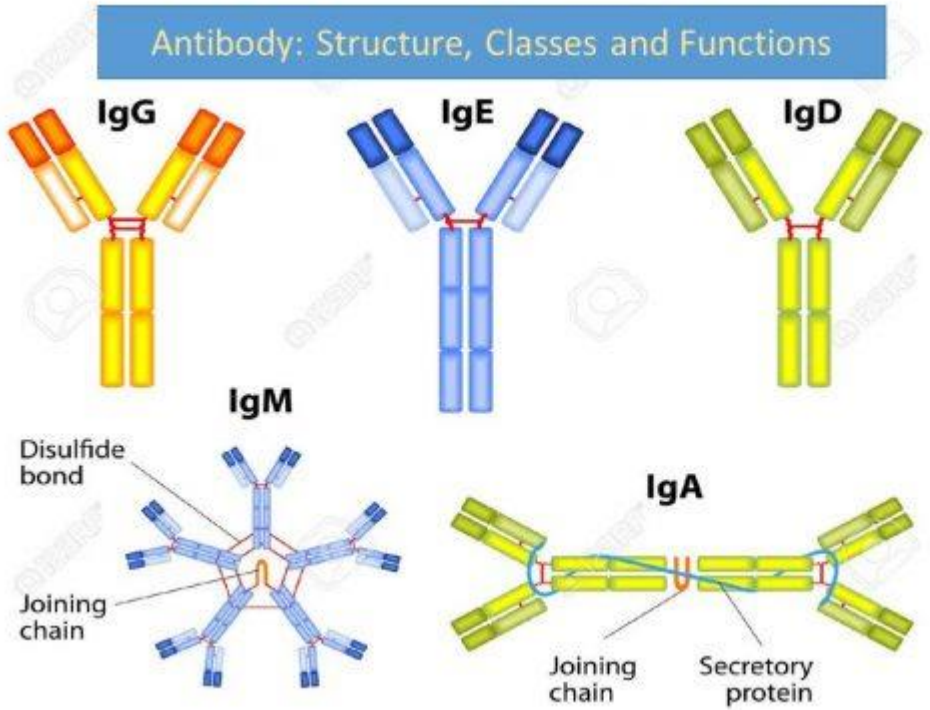
An antibody is a protein component of the immune system that circulates in the blood, recognizes foreign substances like bacteria and viruses, and neutralizes them. After exposure to a foreign substance, called an antigen, antibodies continue to circulate in the blood, providing protection against future exposures to that antigen.



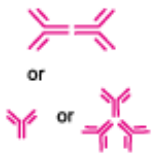


https://www.genome.gov/genetics-glossary/Antibody

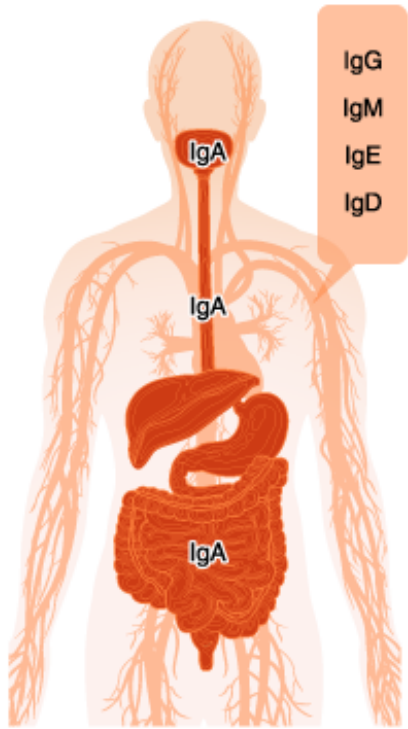
Types of Abs



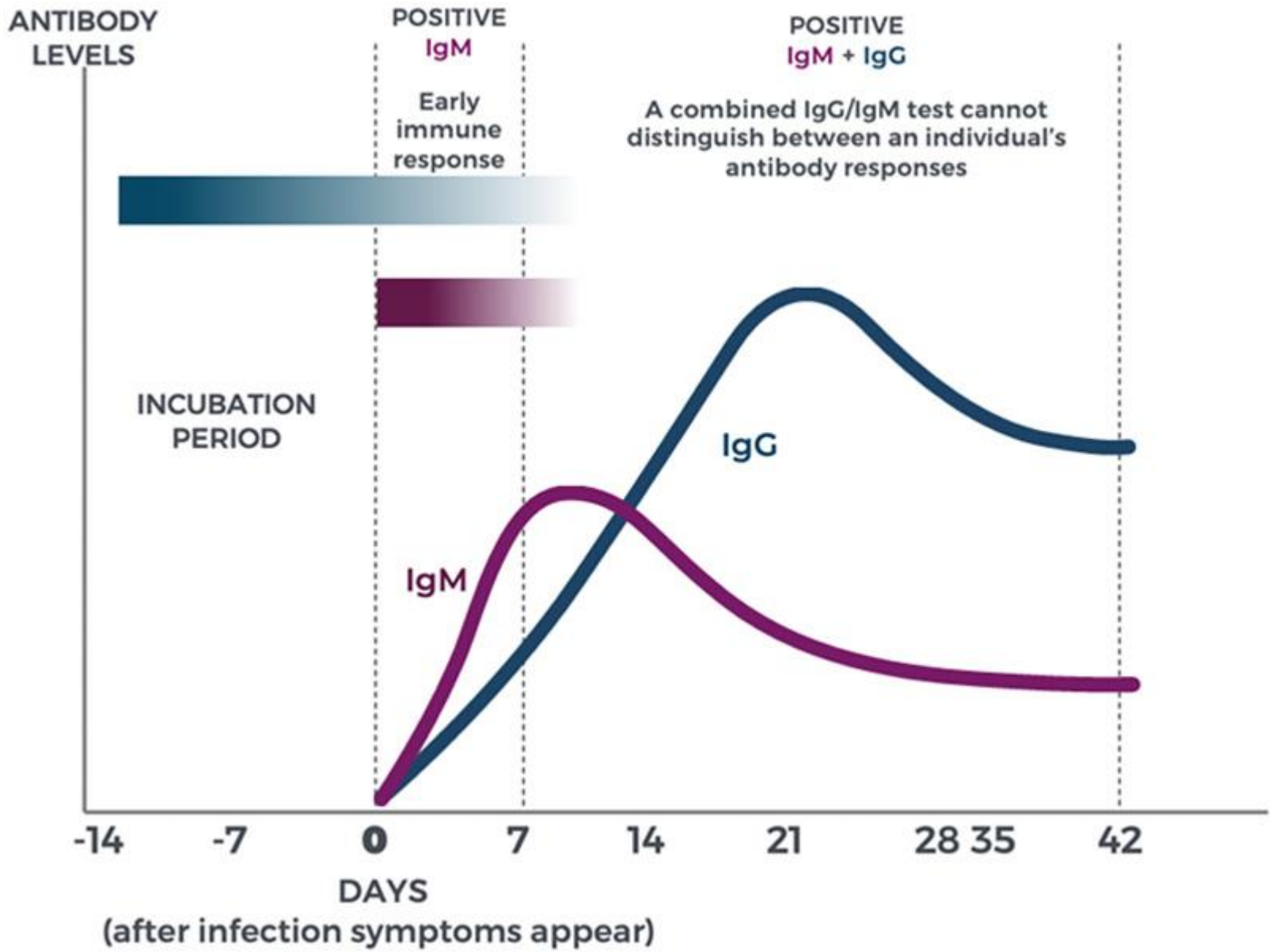
Types and characteristics of antibodies

IgG		• Highest opsonization and neutralization activities. • Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4).
IgM		• Produced first upon antigen invasion. Increases transiently.
IgA		• Expressed in mucosal tissues. Forms dimers after secretion.
IgD		• Unknown function.
IgE		• Involved in allergy.

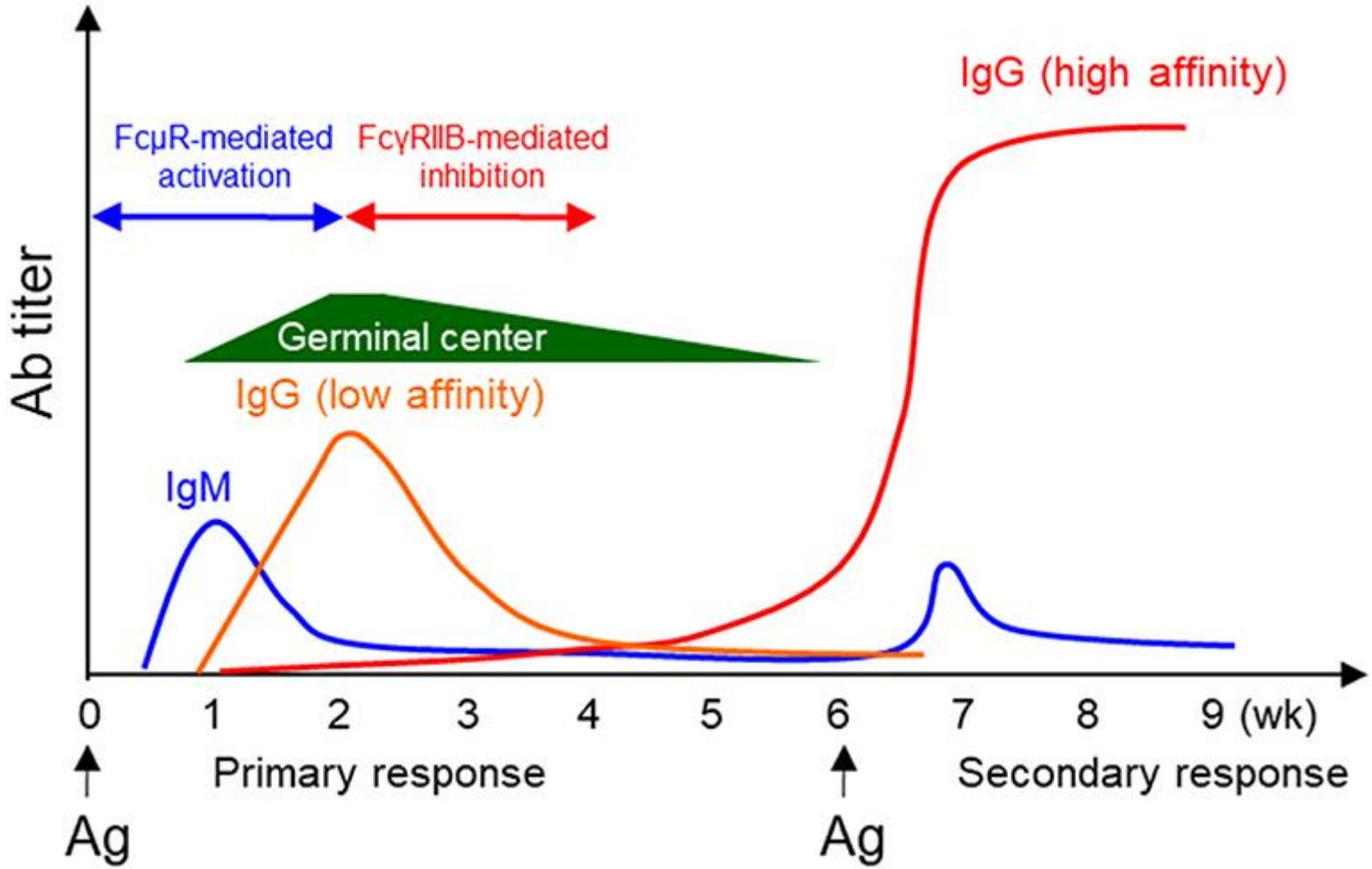
Distribution in the body



https://www.onlinebiologynotes.com/antibody-structure-classes-functions/
https://ruo.mbl.co.jp/bio/e/support/method/antibody-isotype.html



https://www.beckmancoulter.com/en/blog/diagnostics/standalone-igg-vs-total-igm-igg

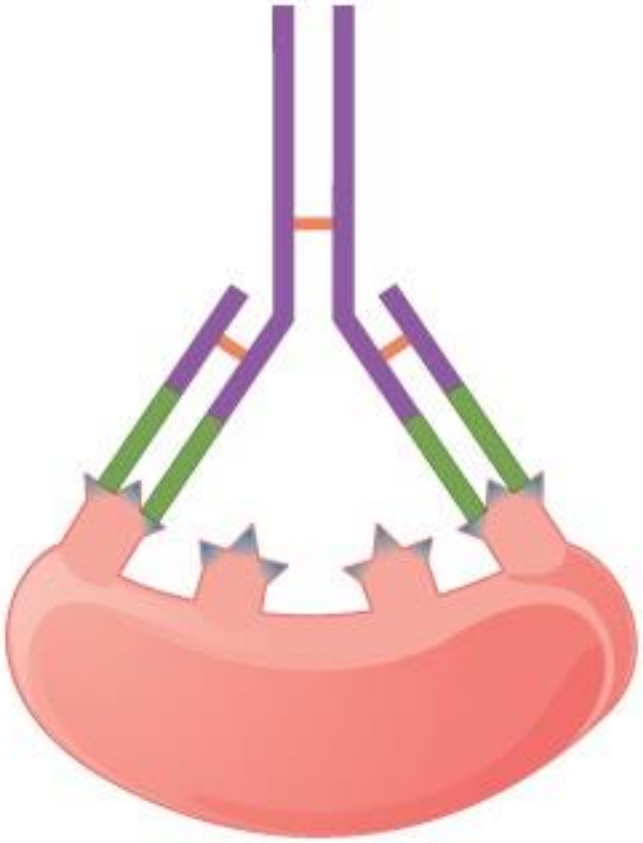

IgG (high affinity)
FcμR-mediated activation
FcγRIIB-mediated inhibition
Germinal center
IgG (low affinity)
IgM
Ab titer
0
1
2
3
4
5
6
7
8
9 (wk)
Primary response
Secondary response
Ag
Ag

Affinity vs Avidity

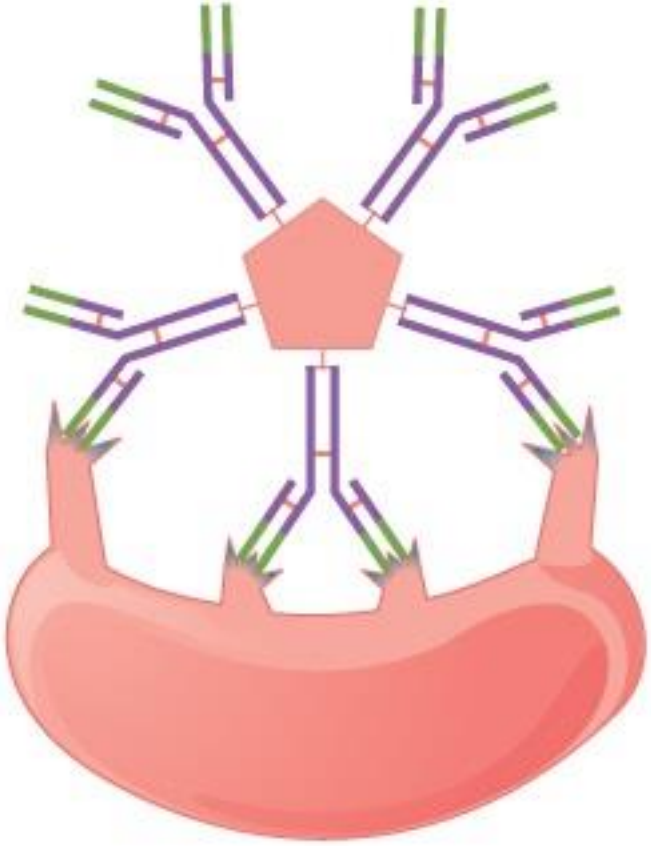
- Affinity: Affinity is the strength of a single bond or interaction. When it comes to the antibody-antigen relationship, the binding affinity is the strength of the interaction between the antigen's epitope and the antibody's paratope at a singular binding site.
- Avidity: Antibodies and antigens are multivalent, meaning they possess more than one binding site. The measure of the total binding strength of an antibody at every binding site is termed avidity.
- Affinity and avidity are both measures of binding strength. While affinity is the measure of the binding strength at a single binding site, avidity is a measure of the total binding strength.
  - Antibodies have between two and ten binding sites. Antibodies with fewer binding sites tend to have high affinity and low avidity, while those with greater binding sites tend to have low affinity and high avidity.
- Although IgM has a lower affinity for antigens than IgG, it has higher avidity for antigens because of its pentameric/hexameric structure.

https://www.technologynetworks.com/immunology/articles/affinity-vs-avidity-333559

(a) Affinity versus avidity



Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.

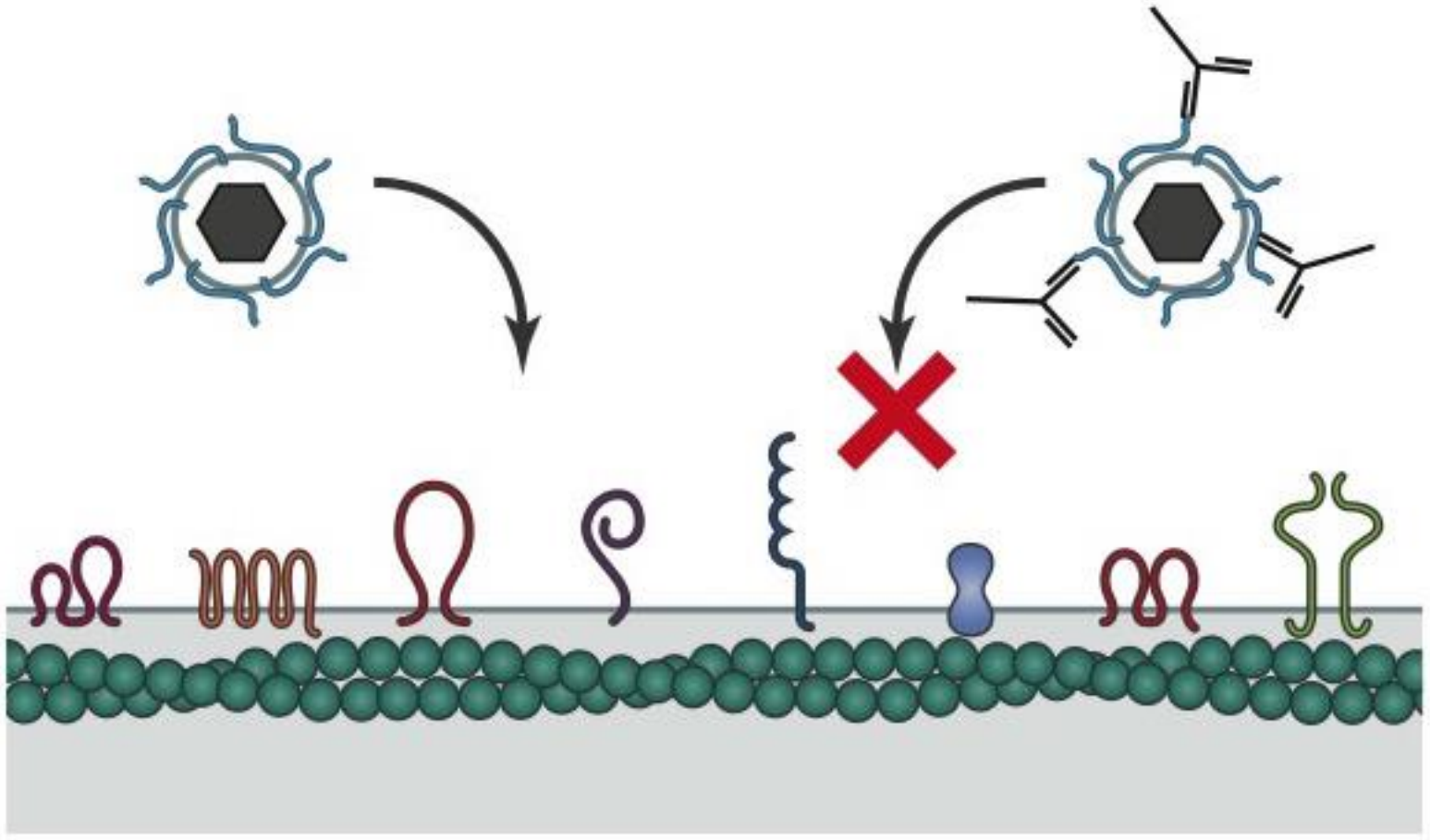


Avidity refers to the strength of all interactions combined. IgM typically has low affinity antigen binding sites, but there are ten of them, so avidity is high.

- Viruses can also be removed from the body by **antibodies** before they get the chance to infect a cell. Antibodies are proteins that specifically recognize invading pathogens and bind (stick) to them. This binding serves many purposes in the eradication of the virus:

1. Firstly, the antibodies neutralize the virus, meaning that it is no longer capable of infecting the host cell.
2. Secondly, many antibodies can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.
3. A third mechanism used by antibodies to eradicate viruses, is the activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the surface of phagocytic cells and triggers a mechanism known as **phagocytosis**, by which the cell engulfs and destroys the virus.
4. Finally, antibodies can also activate the complement system, which opsonises and promotes phagocytosis of viruses. Complement can also damage the envelope (phospholipid bilayer) that is present on some types of virus

- **Neutralization:** It is the blocking of infectivity of a virus by antibodies, that are homolog to this virus, in in-vivo, and in vitro systems.
  - It is the loss of **virus** infectivity caused by antibody.



What for do we perform Neutralization?

- In epidemiological controls
- to diagnose a clinical infection
- Control, follow-up and eradication of certain diseases in a herd
- In quarantine centers
- Antibody detection before and after vaccination
- To Identificate the virus isolates

Positive serum: It is the serum known to have antibody (Ab) against a specific agent (a specific virus, for example BVDV). Positive serum can carry Ab against this virus along with Ab against other agents.

Negative Serum: It is a serum that does not have Ab against a specific agent (a specific virus, for example, BVDV). It may or may not carry Ab against other agents.

Hyperimmune serum: It is a serum which is obtained by giving a specific virus to a sensitive host within the scope of a certain protocol and carrying Ab in a high titer against this virus.

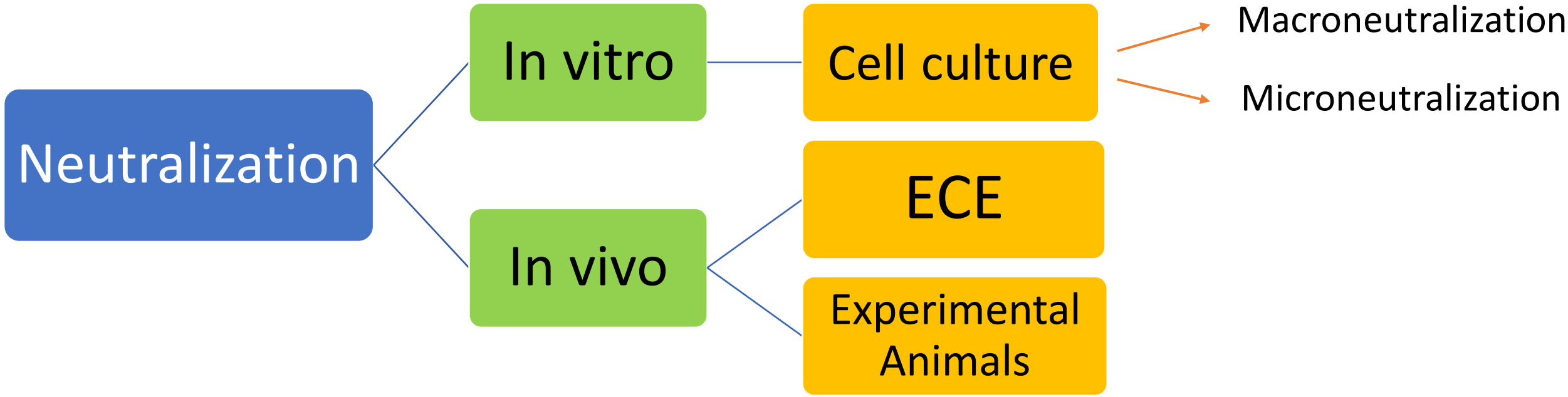
Type-specific serum: A positive serum containing Ab against one of the serotypes or subtypes of a particular agent. For example; Positive serum containing Ab against one of the serotypes (A, O, C, SAT1, SAT2, SA3, Asia1) of foot and mouth disease virus.

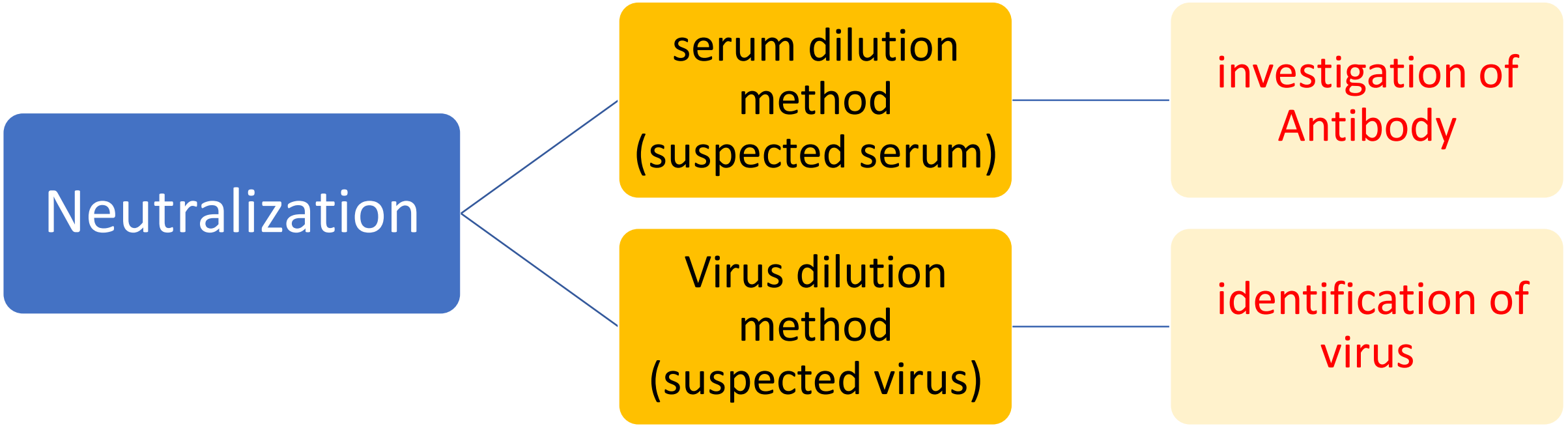
Polyclonal Antibody: It is the serum containing Ab against multiple (usually all of them) antigenic determinants of a virus.

Monoclonal Antibody: It is the antibody showing SPECIFICITY to a SINGLE antigenic determinants of a virus.

Seroconversion: It is the transition of an animal from seronegative to seropositive in terms of antibodies specific to pathogen. This situation may develop due to vaccination or infection.

Seroreversion: It is the transition of an animal from seropositive to seronegative in terms of antibodies specific to pathogen.


Neutralization
In vitro
In vivo
Cell culture
ECE
Experimental Animals
Macroneutralization
Microneutralization


Neutralization
serum dilution method (suspected serum)
Virus dilution method (suspected virus)
investigation of Antibody
identification of virus

Microneutralization

A. Serum dilution method (suspected serum, known virus)

- investigation of Antibody

B. Virus dilution method (suspected virus, known Ab (serum))

- identification of virus

A. Serum dilution method (suspected serum, known virus)

1. Serum Neutralization

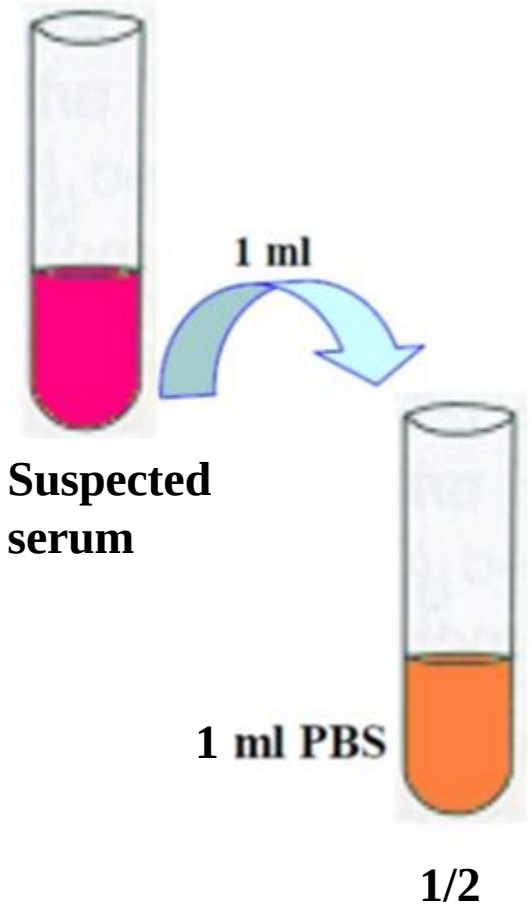
- Is there any Antibody in the suspected serum?

2. SN50 Test

- How much is the Antibody amount in the positive serum?

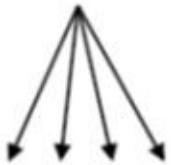
A. Serum dilution method (suspected serum, known virus)

- Purpose: To find out if there is antibody !!!



1 ml suspected serum+1 ml PBS

1/2



0.05 ml

The virus diluted at the rate of $100TCID_{50}$ ($100TCID_{50}$ virus) is added to all wells 0.05 ml.

CC: Cell Control

- 0.1 ml Earle with sera

VC: Virus control

- 0.05 ml VIRUS
- 0.05 ml Earle without sera

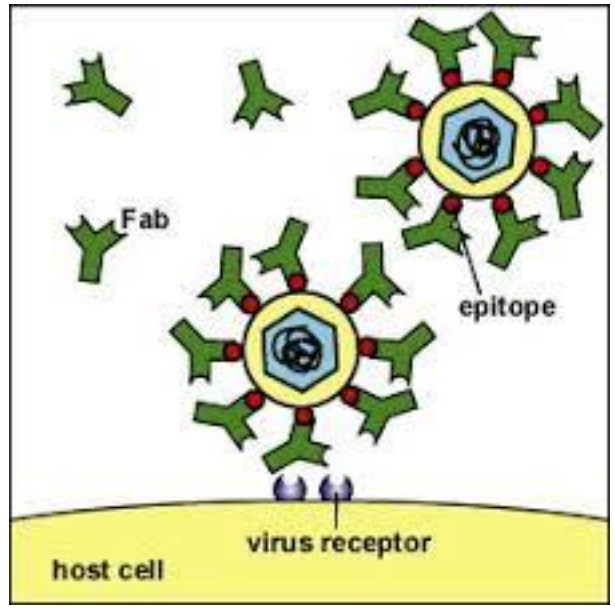
½ serum + 100$TCID_{50}$ virus

CC

VC

- 1 h in 37℃ incubation

37℃ and %5 CO2 incubator



Finally, 0.05 ml (1 DROP) cell suspension to ALL WELLS (BY DROPPER!)

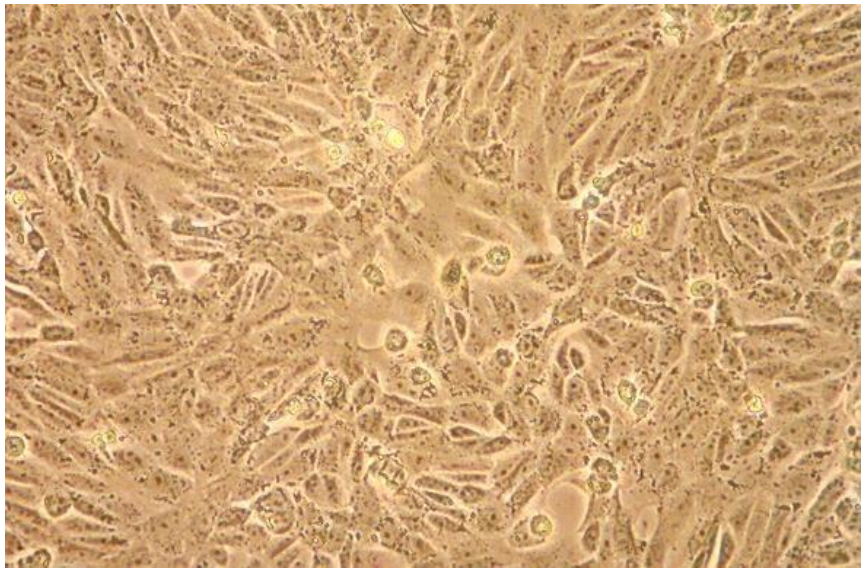
- Test is over

37°C ve %5 CO2 incubator

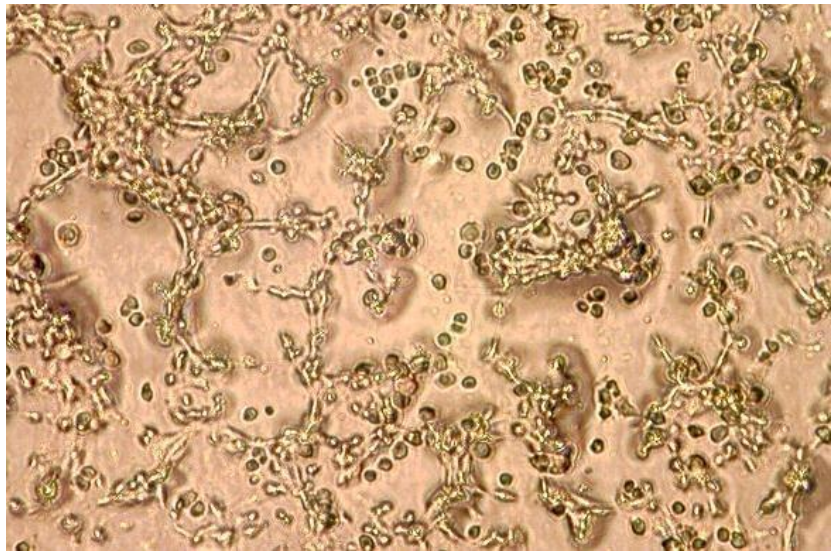
We examine it every day under an invert microscope.

CPE

Suspected serum	CPE	Neutralization
If there is Ab (homologous) against the tested virus	(-)	(+)
If there is no Ab (homologous) against the tested virus	(+)	(-)



CPE(-) Neutralization(+)



CPE(+) Neutralization(-)

- How much is the Antibody amount in the positive serum?

SN_{50} Detection in Positive Serum

SN_{50}

It is serum dilution that blocks virus replication in half of cell cultures infected with 100$TCID_{50}$ diluted virus.

½ diluted serum is transferred to the first 4 wells at a rate of 0.1 ml.

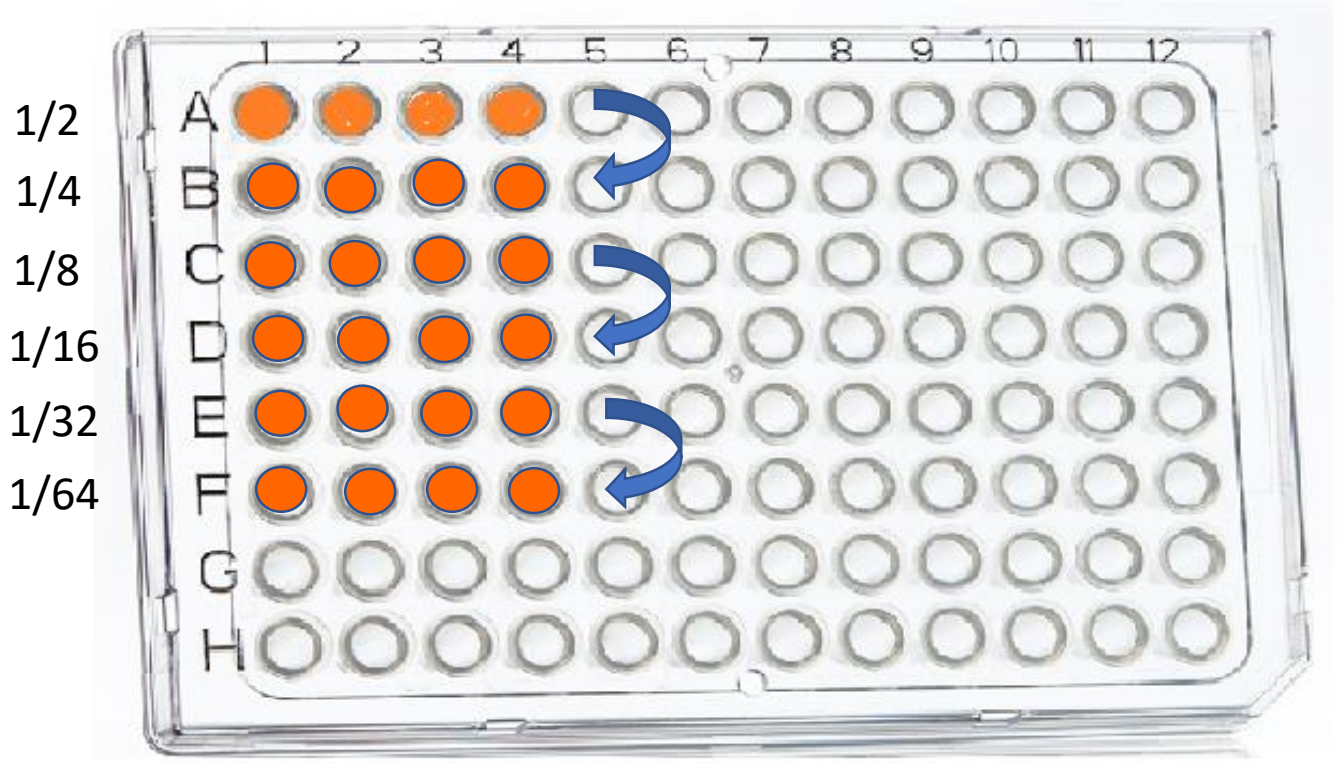
0.05 ml of PBS is instilled into the other wells (BY DROPPER).

Or

After placing 0.05 ml of PBS (BY DROPPER) into all wells, 0.05 ml of serum is put into the first 4 wells.

1/2

- The serum, which is taken from the first wells (A), with the help of diluter, is transferred to other wells and diluted of the serum according to the **log_2** base.



- The virus diluted at the rate of 100$TCID_{50}$ is added 0.05 ml into all wells.

CC: Cell Control

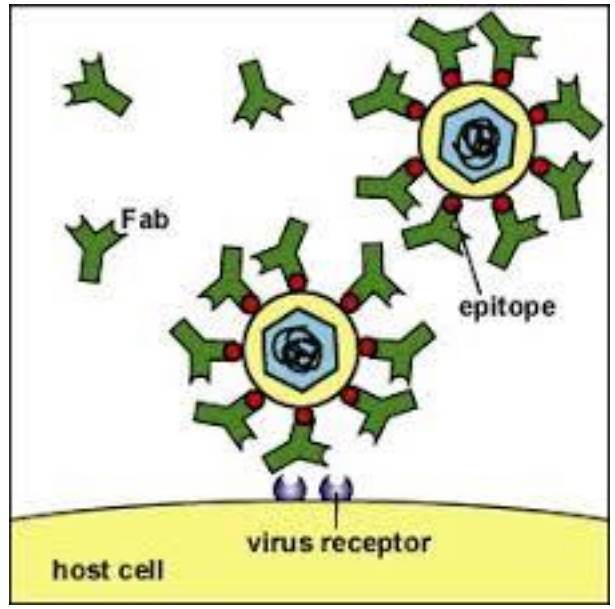
- 0.1 ml Earle with sera

VK: Virus Control

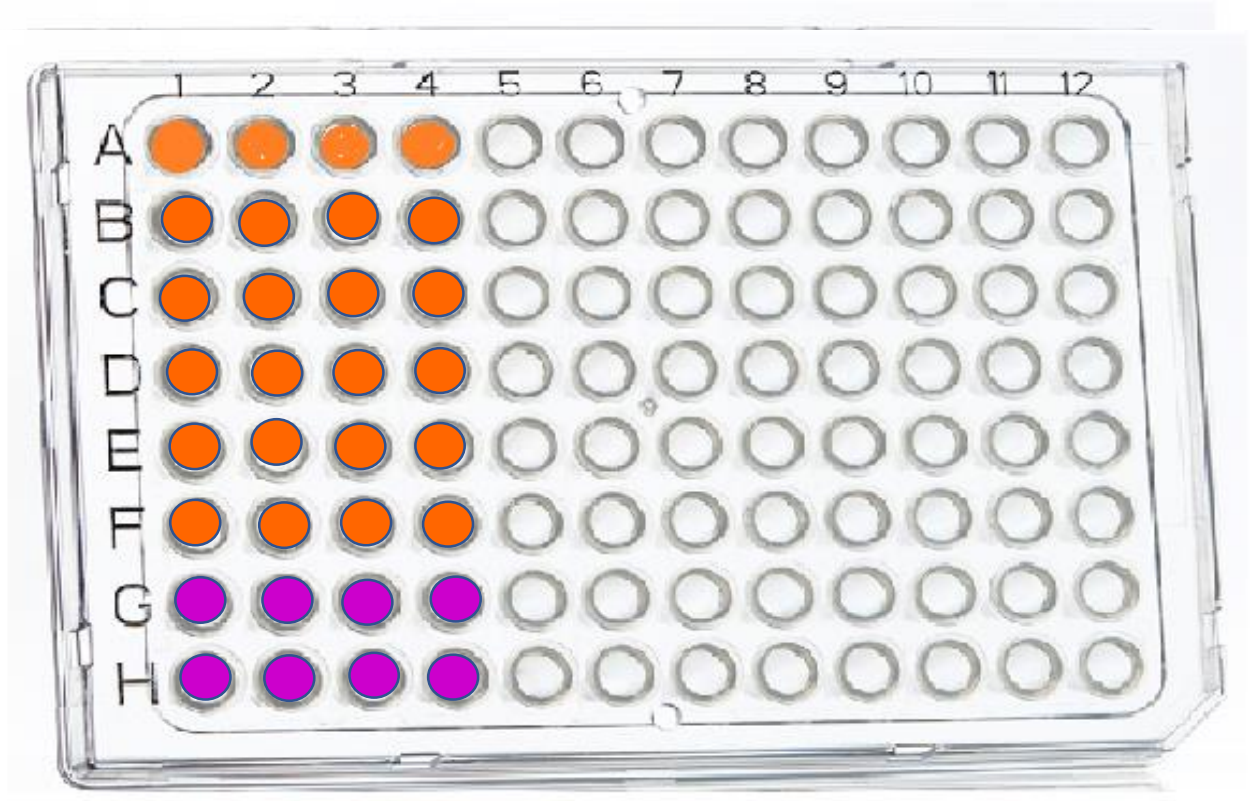
- 0.05 ml VİRUS
- 0.05 ml Earle without sera

- 1 h in 37℃ incubation

37℃ and %5 CO2 incubator



Finally, 0.05 ml (1 DROP) cell suspension to ALL WELLS (BY DROPPER!)



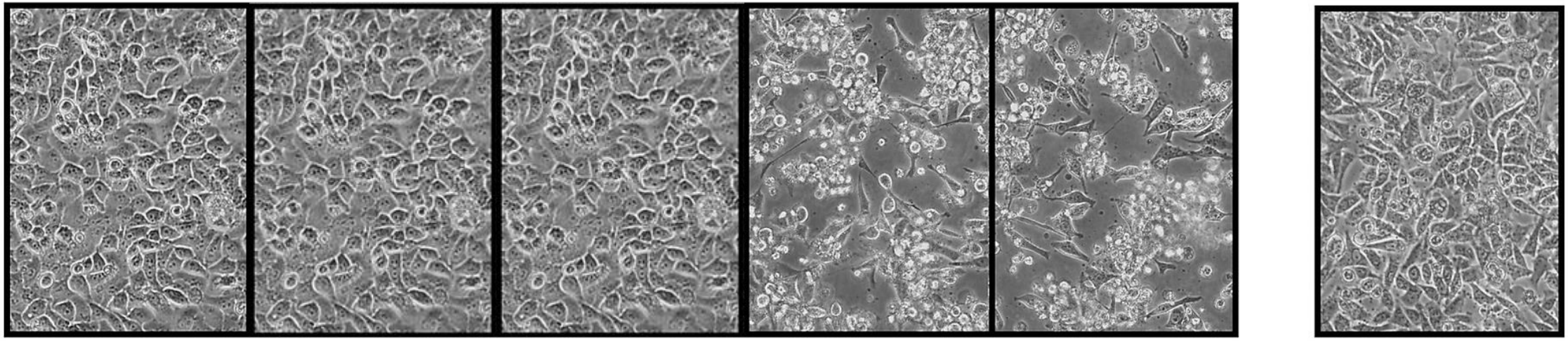
- Test is over

37°C ve %5 CO2 incubator

We examine it every day under an invert microscope.

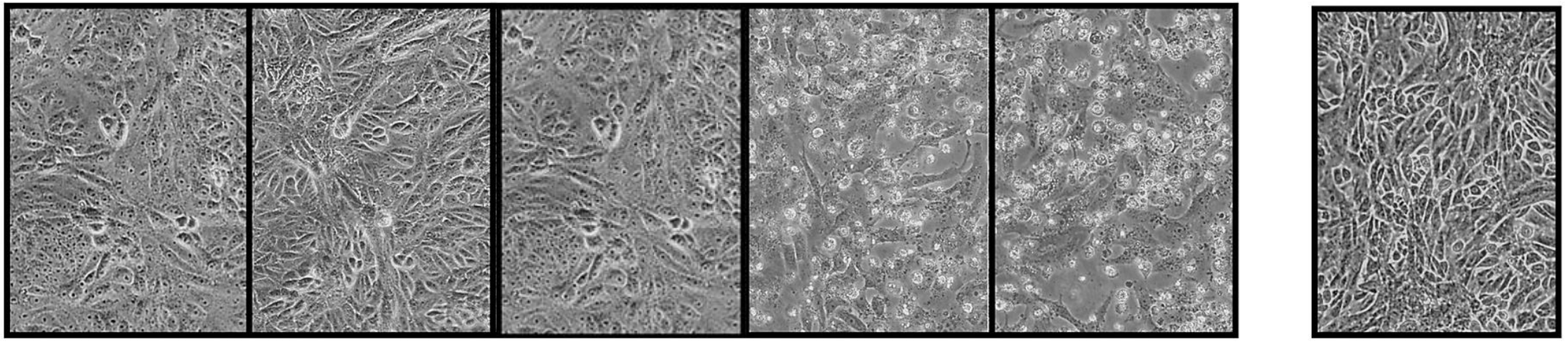
CPE

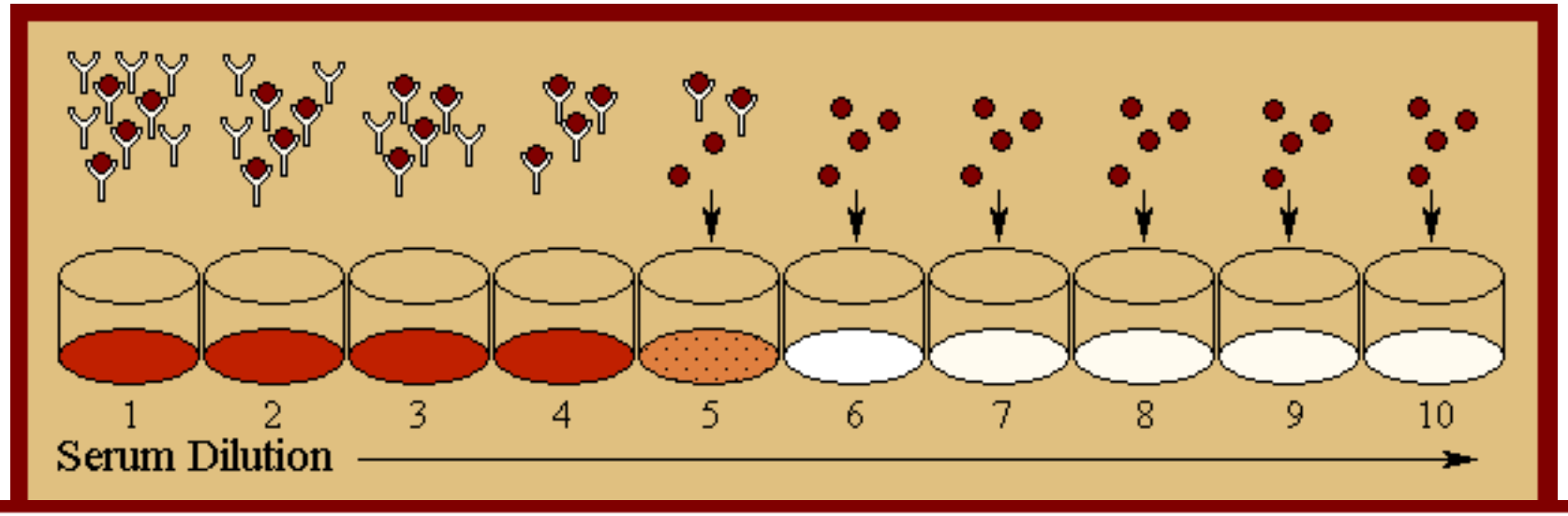
1/2	1/4	1/8	1/16	1/32	Cell control



CPE increases !!!!!!

1/2	1/4	1/8	1/16	1/32	cell control





serum sulandırmaları	CPE	Nötralizasyon
1/2	0/4	+
1/4	0/4	+
1/8	0/4	+
1/16	0/4	+
1/32	2/4	+
1/64	4/4	-
HK	0/4	
VK	4/4	

B. Virus dilution method (suspected virus, known Ab (serum))

- Purpose: to identify the virus!!!

Suspected virus + known serum
(100DKID50) (SN50)

CC

VC

CC: Cell Control

- 0.1 ml Earle with sera

VC: Virus control

- 0.05 ml VIRUS
- 0.05 ml Earle without sera