Hemagglutination (HA) Assay

The hemagglutination assay is a method for titering viruses (mostly influenza) based on their ability to attach to molecules present on the surface of red blood cells.

HA-red blood cell relationship

The ability of the virus to hemagglutinate is limited to the species from which erythrocytes are obtained.

Preparation of erythrocyte suspension

- Anticoagulated blood centrifuged for 5-10 minutes.

at 2000 rpm

-After centrifugation, the plasma and leukocyte layer is discarded, and the erythrocytes remaining in the bottom of the tube are washed 3 times with 0.85% FTS or PBS solution.

-After the last wash, the erythrocytes inside the tube are accepted as 100% and diluted to 0.5-1% according to the purpose.

-The erythrocyte suspension can be stored at + 4 ° C for up to 1 month.

Hemagglutination is done in 2 forms according to purpose.

- **1.** Quick HA:
- Qualitative evaluation can be done.
- It is understandable whether or not the virus has HA ability and which species have erythrocytes HA.

HA titres can not be determined.

2. Slow HA:

It is suitable for quantitative evaluation. The titer of the virus is determined.

Rapid HA test

- The virus (?) And erythrocyte are mixed with a drop on a slide and the result is evaluated after 3-5 minutes in room temperature.
- **Result:** reddish color HA
- (+) (+)
- (-) (-)

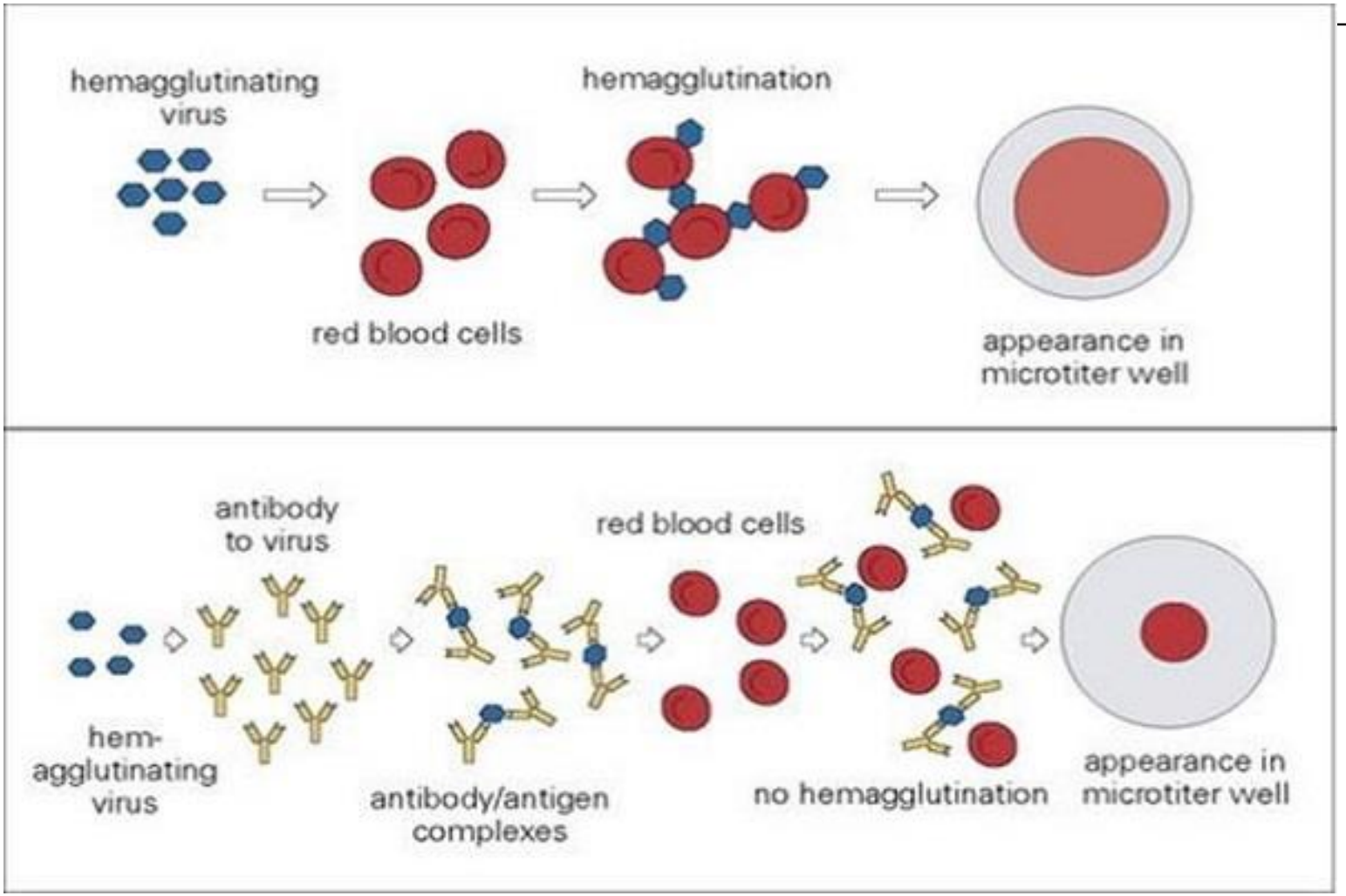
SLOW HA assay

- Principle;
- Log 2 fold virus dilutions is combined with an appropriate volume of the erythrocyte suspension in the HA plate, and after the appropriate incubation period, the HA is formed or the dilution step is determined.

Evaluation of HA test;

- GRID (reddish) style image HA (+)
- BUTTON (dot)-style display HA (-)

http://www.microbiologynotes.com/haemagglutination-assay-viral-quantitation/



Determination of HA titre (HB)

- **HB:** The last dilution step that agglutinates the same volume of erythrocytes
- dilution HA
- **1/2** +
- 1/4 +
- 1/8 +
- 1/16 – HB: 1/8

Usage areas of HA test

- Identification of an isolated virus
- Standardization of virus to be used for HI test

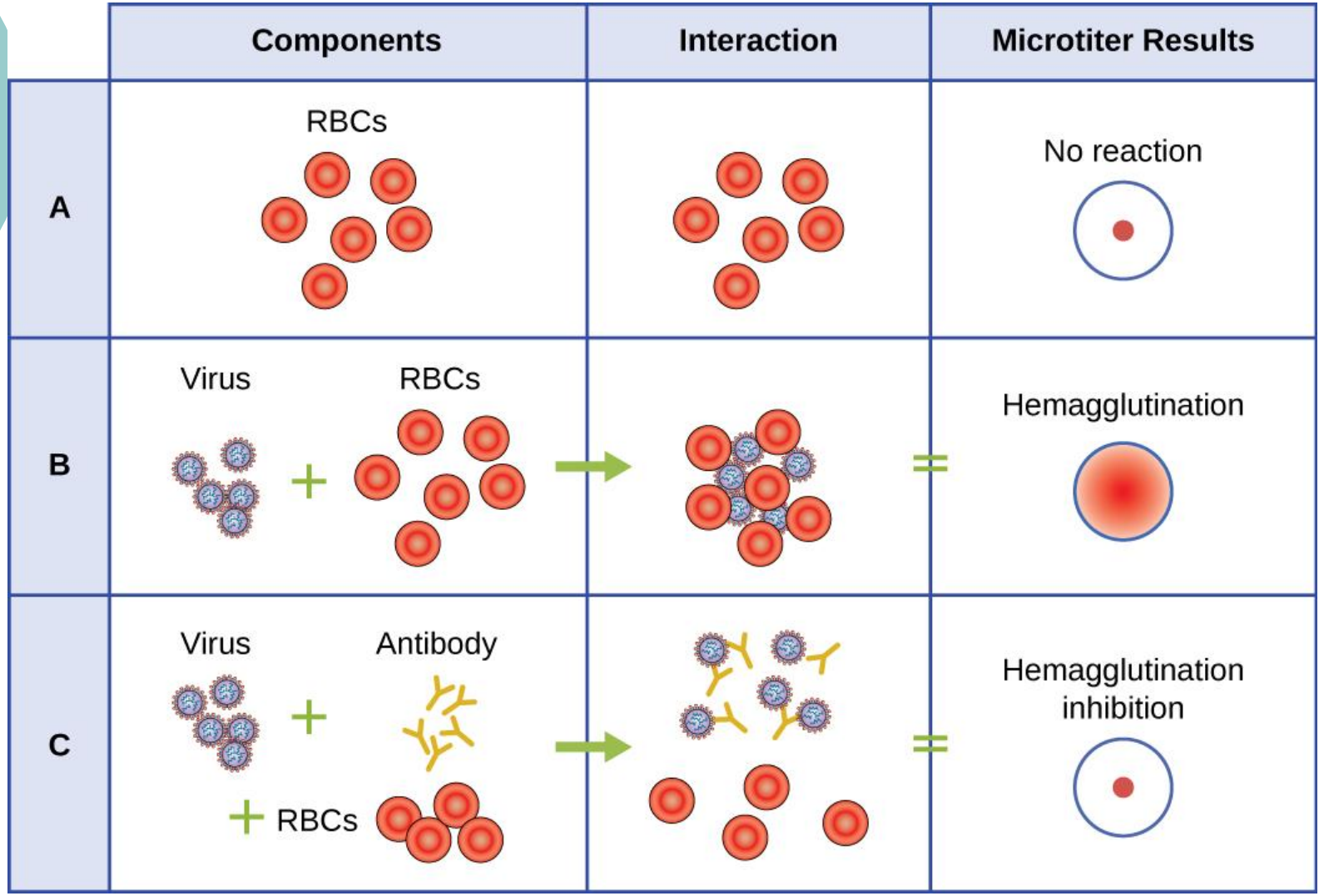
INDIRECT HAEMAGGLUTINATION ASSAY (IHA)

- The indirect haemagglutination assay (IHA) can be used to detect antibodies.

The HI test is applied for 2 purposes.

- 1. Identification of antigen (virus dilution method)
- 2. Detect antibody presence and titre in suspected serum (serum dilution method)

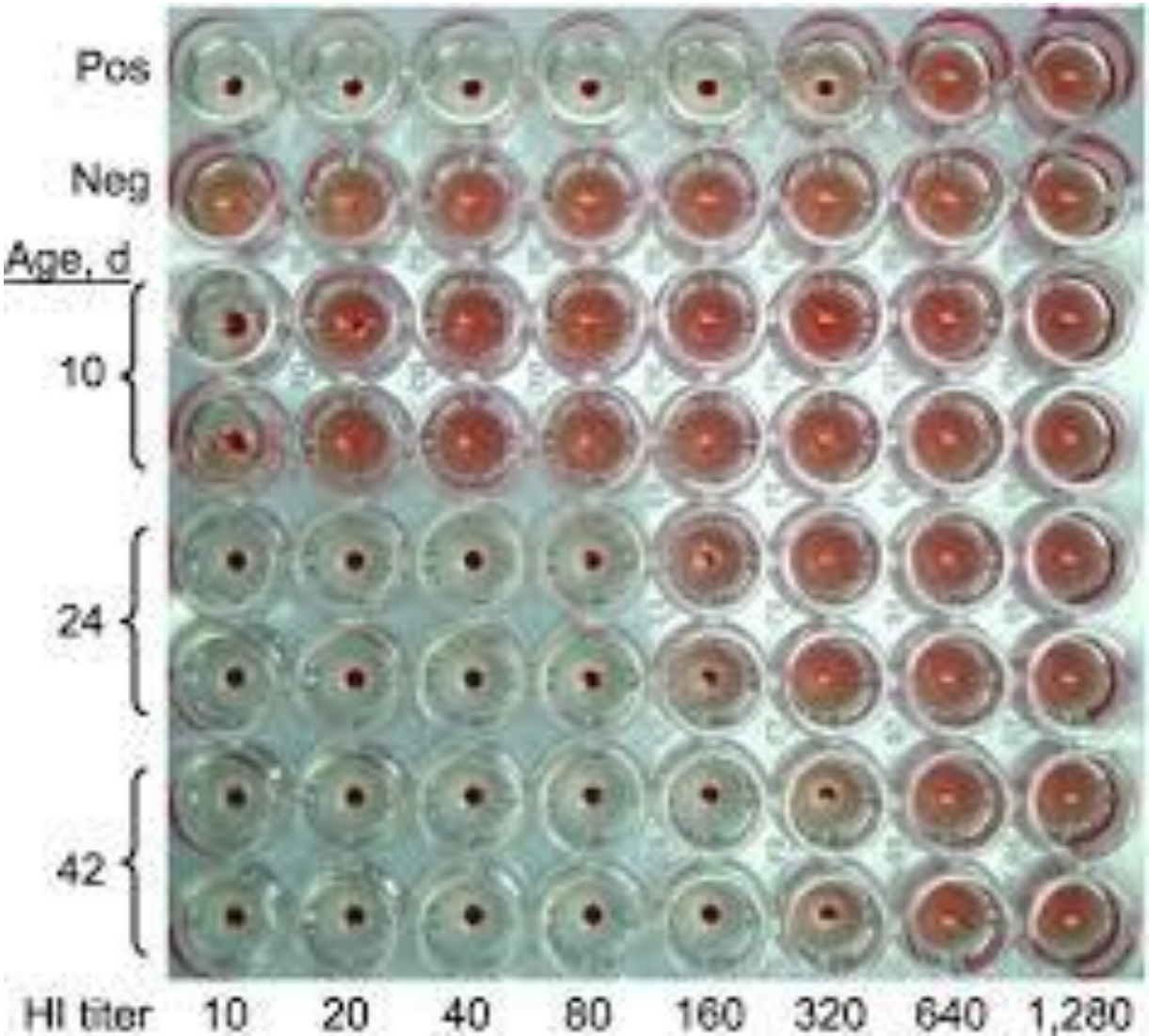
https://www.quizover.com/microbiology/section/nucleic-acid-amplification-test-by-openstax
modification of work by Centers for Disease Control and Prevention



Hemagglutination Inhibition Test

Perinatal pandemic (H1N1) 2009 infection, Thailand.

Dulyachai W, Makkoch J, Rianthavorn P, Changpinyo M, Prayangprecha S, Payungporn S, Tantilertcharoen R, Kitikoon P, Poovorawan Y - Emerging Infect. Dis. (2010)



VIRUS IDENTIFICATION
HI TEST

- Assessing the result by incubating the same amount of serum known to be suspicious with the appropriate volume of the tube in the plate, and then adding the erythrocyte to the medium.

Evaluation of virus dilution

- The suspicious virus and serum homologues bind antigen-antibody and HA does not occur because antigen-erythrocyte binding does not occur. In this case, HI (+) is.
- Virus (?) - Antibody HA HI
- Homolog (-) (+)
- Not Homologous (+) (-)

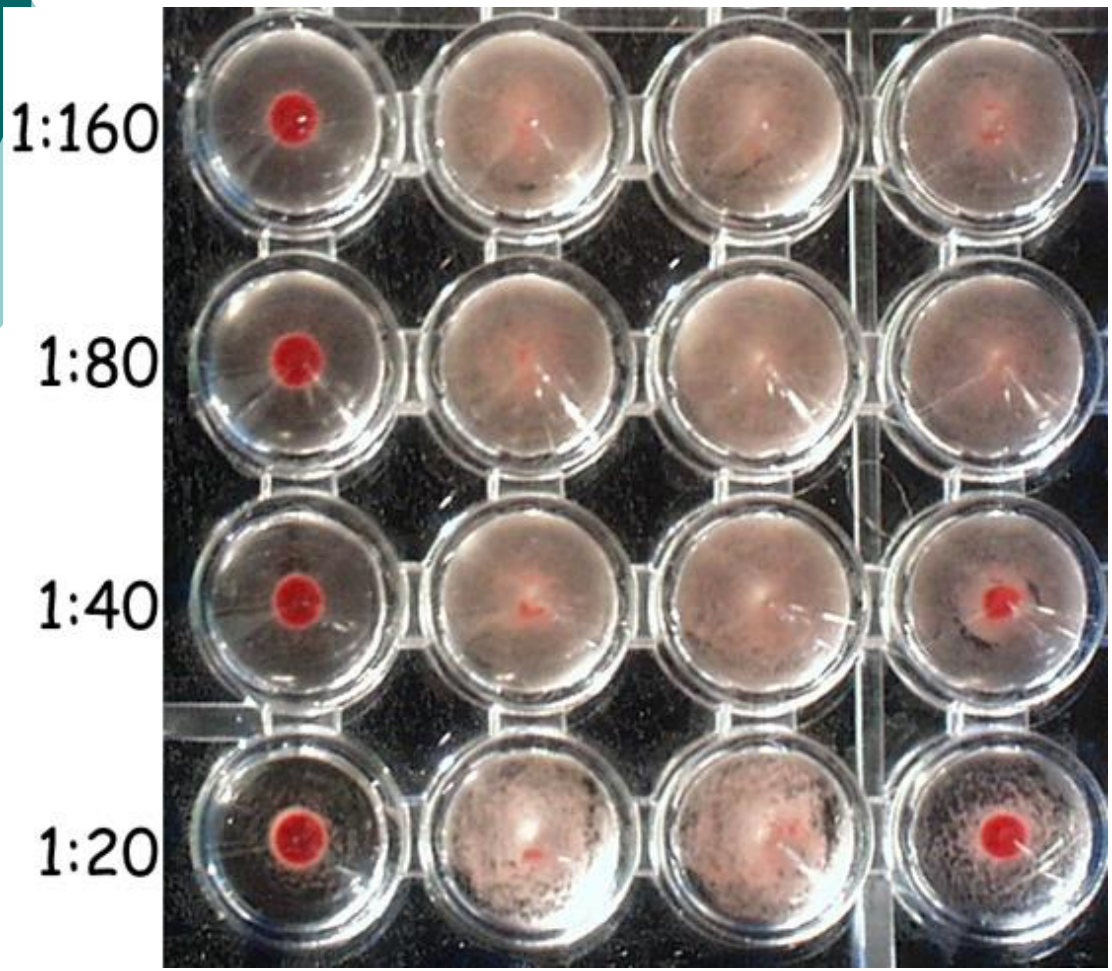
HI TEST FOR ANTIBODY DETECTION

- Is the evaluation of the result, by incubating the plate in the same volume of suspected serum and antigen diluted at a ratio of 4 HB, and then adding the erythrocyte to the medium.

Evaluation of serum dilution

- If there is known virus-specific antibodies in the serum, the antigen-antibody will bind and HA will not form because antigen-erythrocyte binding does not take place. In this case, HI (+) is.
- Virus-Antibody (?) HA HI
- Homolog (-) (+)
- Not Homologous (+) (-)

Hemagglutination Inhibition Test
Venezuelan Equine Encephalomyelitis Virus (VEE)



From left to right the results of the
serum samples are:

(1) >1:160
(2) negative
(3) negative
(4) 1:20

dilution	HA	HI
1/2	(-)	(+)
1/4	(-)	(+)
1/8	(-)	(+)
/16	(-)	(+)
1/32	(+)	(-)

- HI titre: 1/16 X 4= 1/4