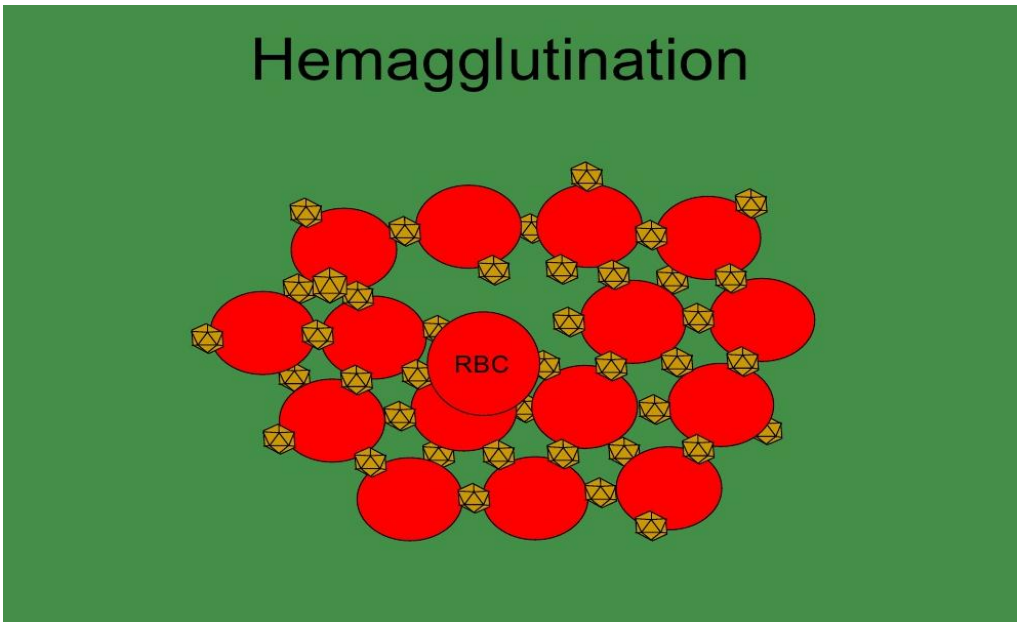
Haemagglutination assay

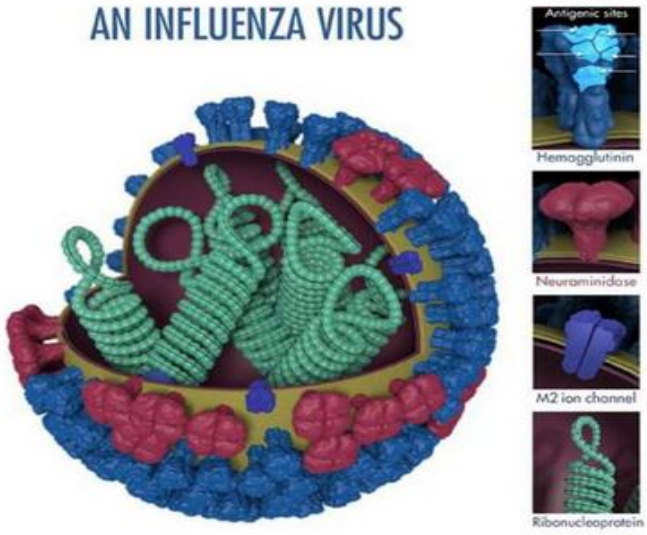
- **Hemagglutination —haemagglutination**, is a specific form of agglutination that involves red blood cells (RBCs).
- It has two common uses in the laboratory: blood typing and the quantification of virus dilutions in a Haemagglutination assay.

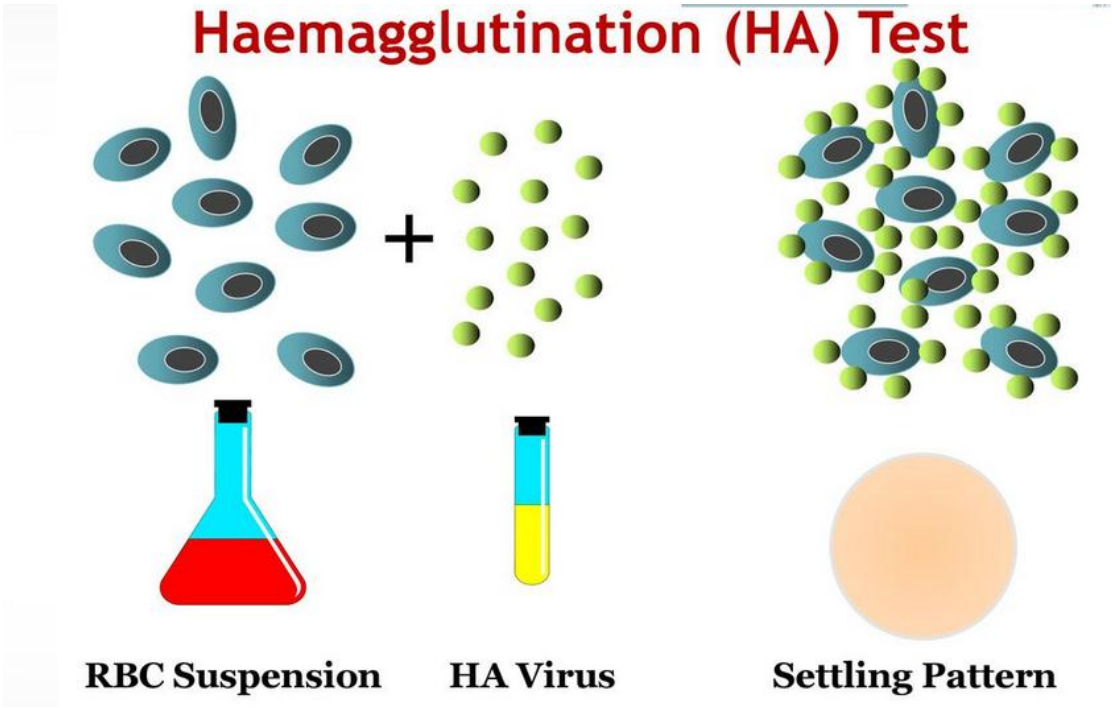


hemagglutination assay

- **Principle**
  - Influenza virus particles have an envelope protein called the hemagglutinin, or HA, which binds to sialic acid receptors on cells.
  - The virus will also bind to erythrocytes (red blood cells), causing the formation of a lattice.
  - This property is called Haemagglutination, and is the basis of a rapid assay to determine levels of influenza virus present in a sample.
  - To conduct the assay, two-fold serial dilutions of a virus are prepared, mixed with a specific amount of red blood cells, and added to the wells of a plastic tray.

Influenza Virus test



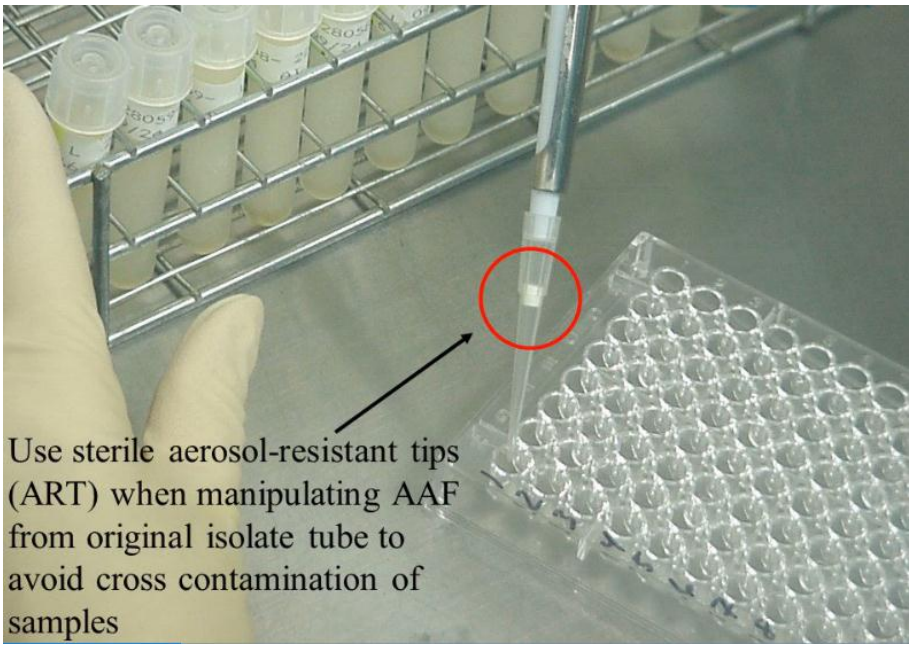


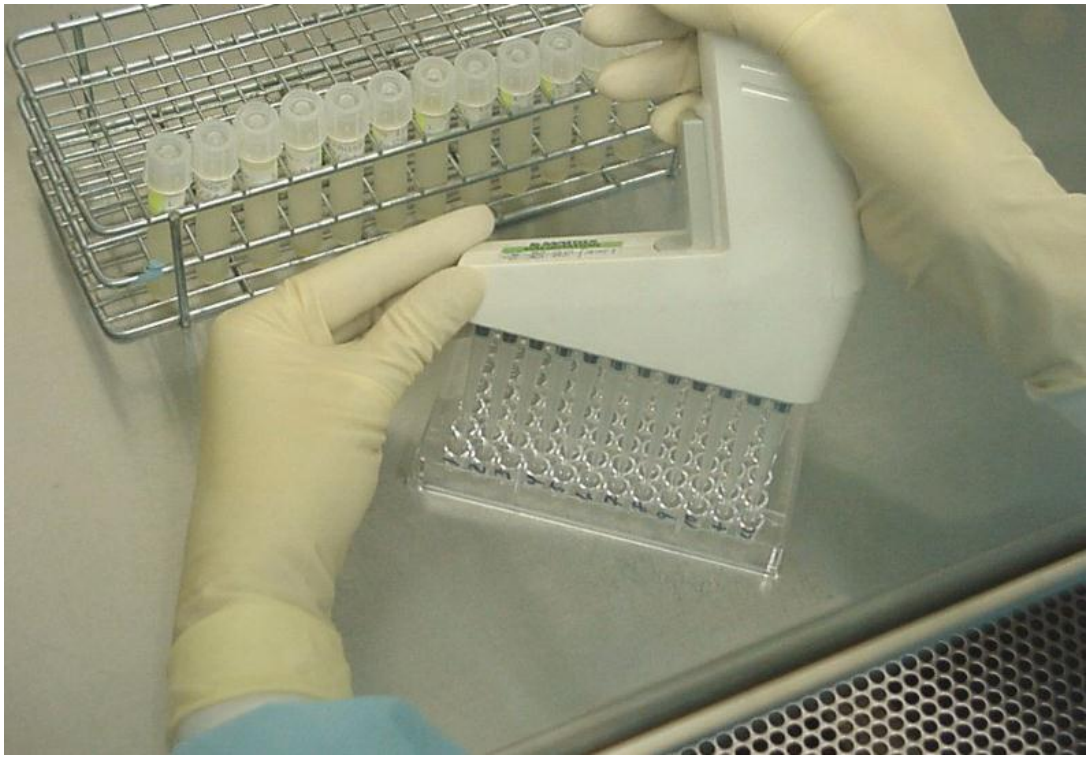
HA/HI Tests
Supplies Needed

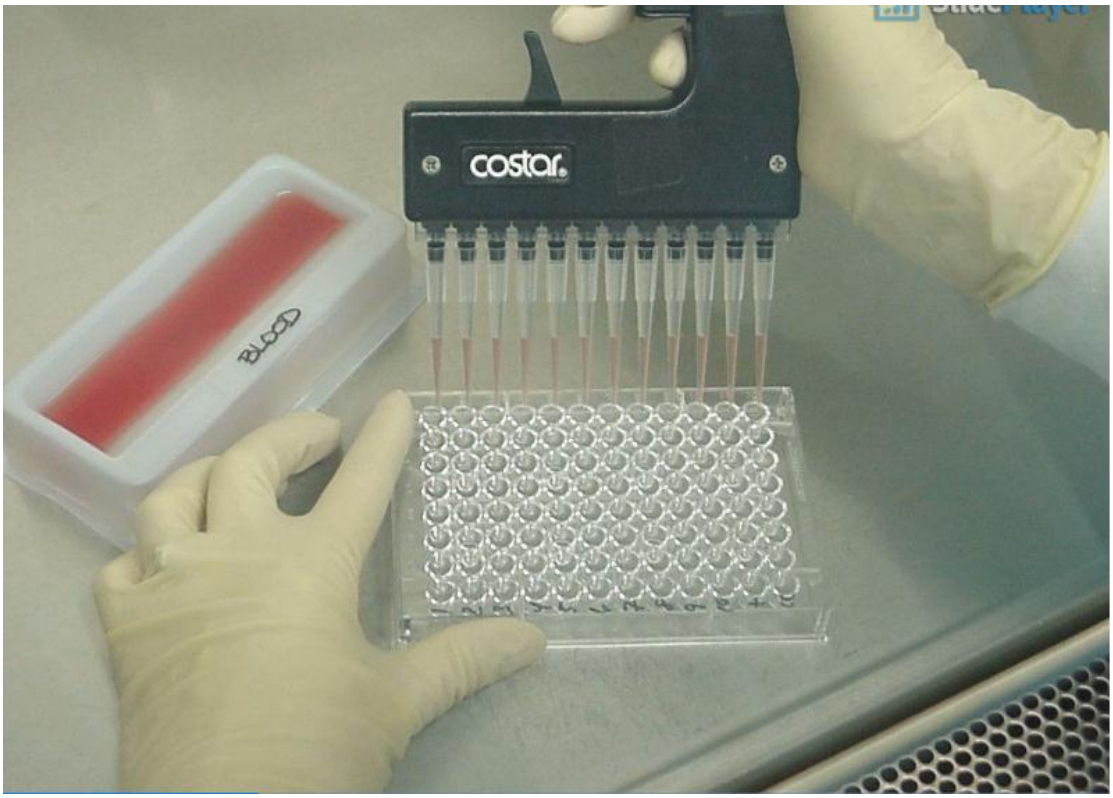
- U- or V-bottom microtiter plates (non-sterile)
- Pipettors with tips (sterile ART, nonsterile)
  - ✓Single channel (calibrated) – 25-200ul
  - ✓Multichannel (calibrated) – 25-200ul
- Phosphate buffered saline (0.01M, pH 7.2)
- Washed chicken erythrocytes (0.5%)

HA Procedure

- Label plates (8 or 12 wells/row)
- Add 50 ul PBS to all wells
- Add 50 ul prepared virus to 1st well
- Mix and dilute (50 ul) - 1st through last well
- Add 50 ul 0.5% washed RBCs to all wells
- Mix by shaking plates
- Read at 20 - 30 minutes (when RBCs buttoned)



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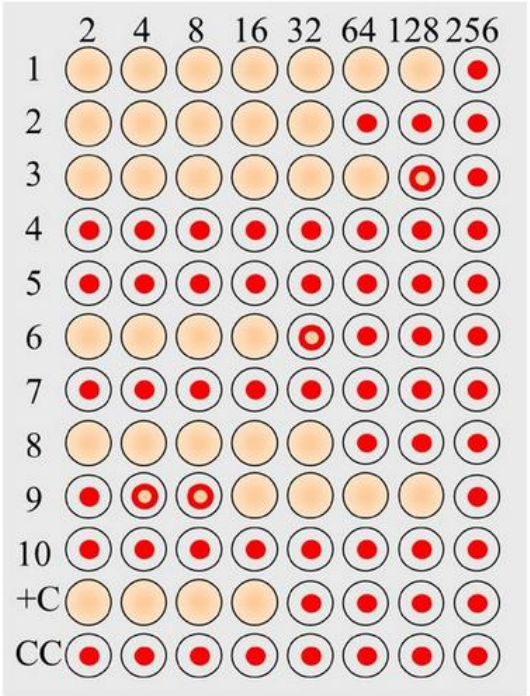
Results



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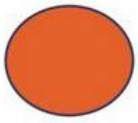
Interpretation

- The red blood cells that are not bound by influenza virus sink to the bottom of a well and form a button.

- The red blood cells that are attached to virus particles form a lattice that coats the well.



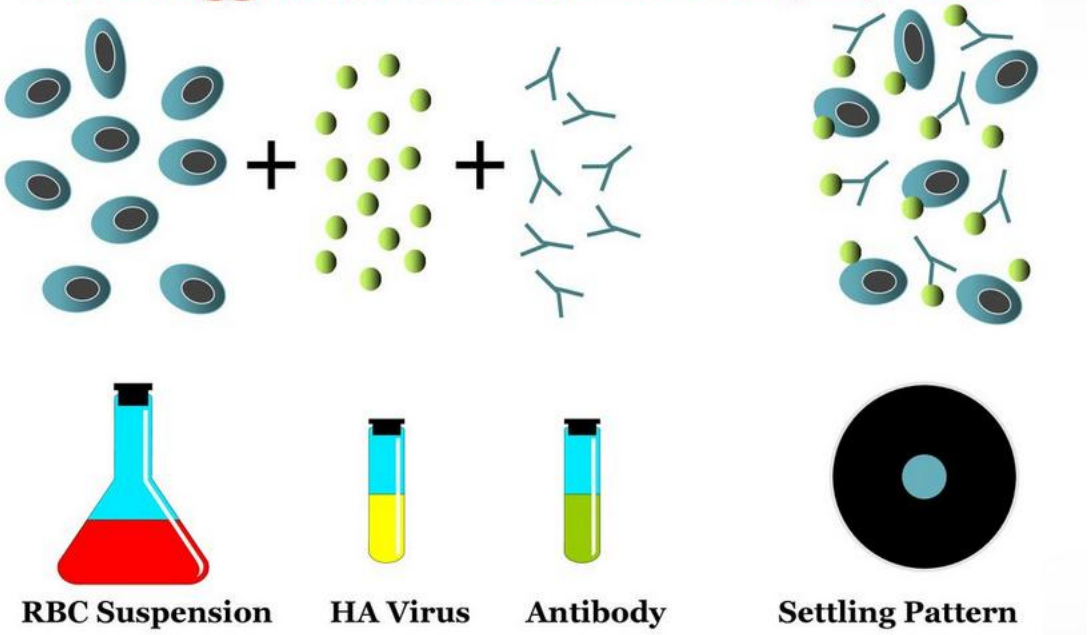
- The assay can be performed within 30 minutes, and is therefore a quick indicator of the relative quantities of virus particles.

Hemagglutination inhibition assay

Principle

- Note that some other viruses and some bacteria will also agglutinate red blood cells. To demonstrate that the haemagglutinating agent is influenza disease virus, it is necessary to use a specific virus antiserum to inhibit the haemagglutinating activity.
- The HA assay can be easily modified to determine the level of antibodies to influenza virus present in serum samples.
- The basis of the HI assay is that antibodies to influenza virus will prevent attachment of the virus to red blood cells.
- Therefore haemagglutination is inhibited when antibodies are present.

Haemagglutination-Inhibition (HI) Test

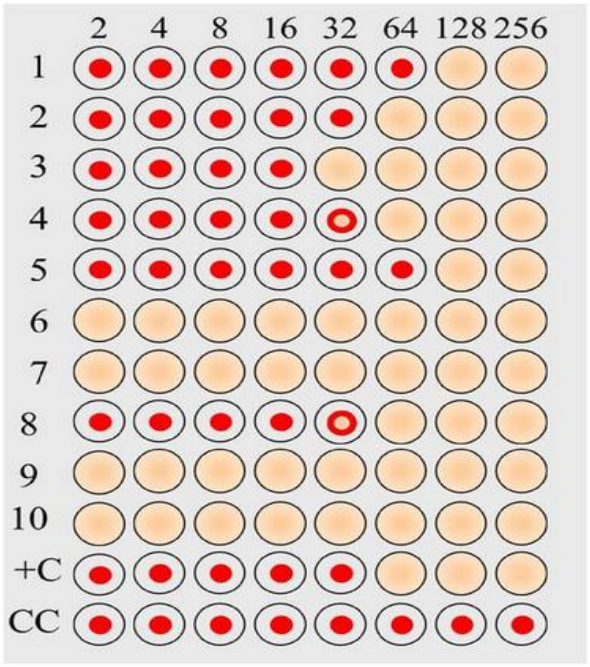


- First obtained a preparation of one of the new influenza viruses, and determined its HA titer by adding a fixed amount of virus to every well of a 96-well plate, equivalent to 32 – 64 HA units.
- Then prepared two-fold dilutions of each serum to be tested, and added each dilution series along a row of wells.
- Finally, added red blood cells and incubated for 30 minutes.

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

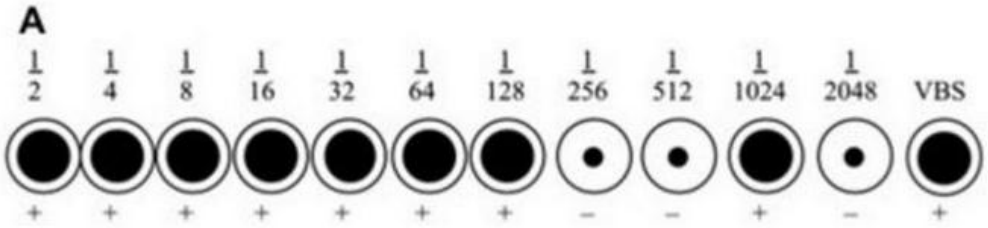
- The highest dilution of serum that prevents haemagglutination is called the HI titer of the serum.
- If the serum contains no antibodies that react with the new strain, then haemagglutination will be observed in all wells.
- Likewise, if antibodies to the virus are present, haemagglutination will not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

Quality Control

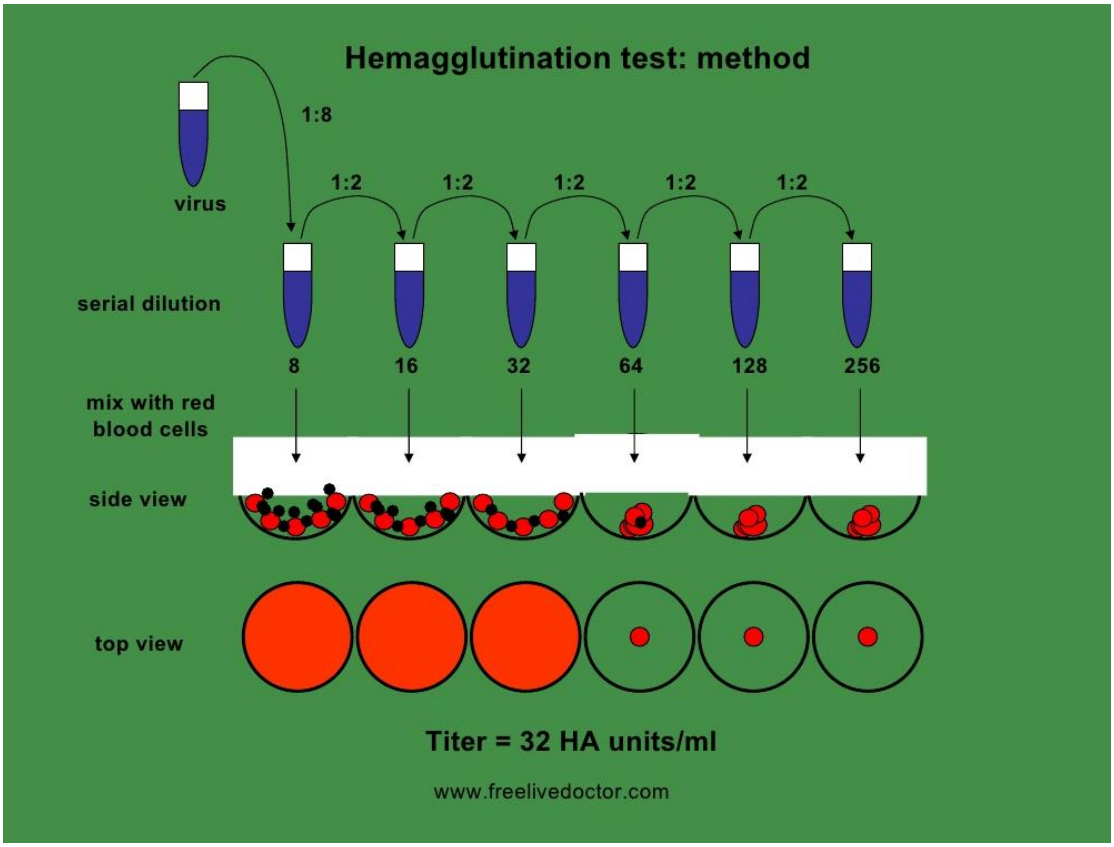
- Known positive serum
- Known negative serum
- Serum and cells without antigen (to detect nonspecific agglutination)
- Back titration of haemagglutination activity of the antigen (to ensure that 4 hemagglutinating virus (HAU) were tested)

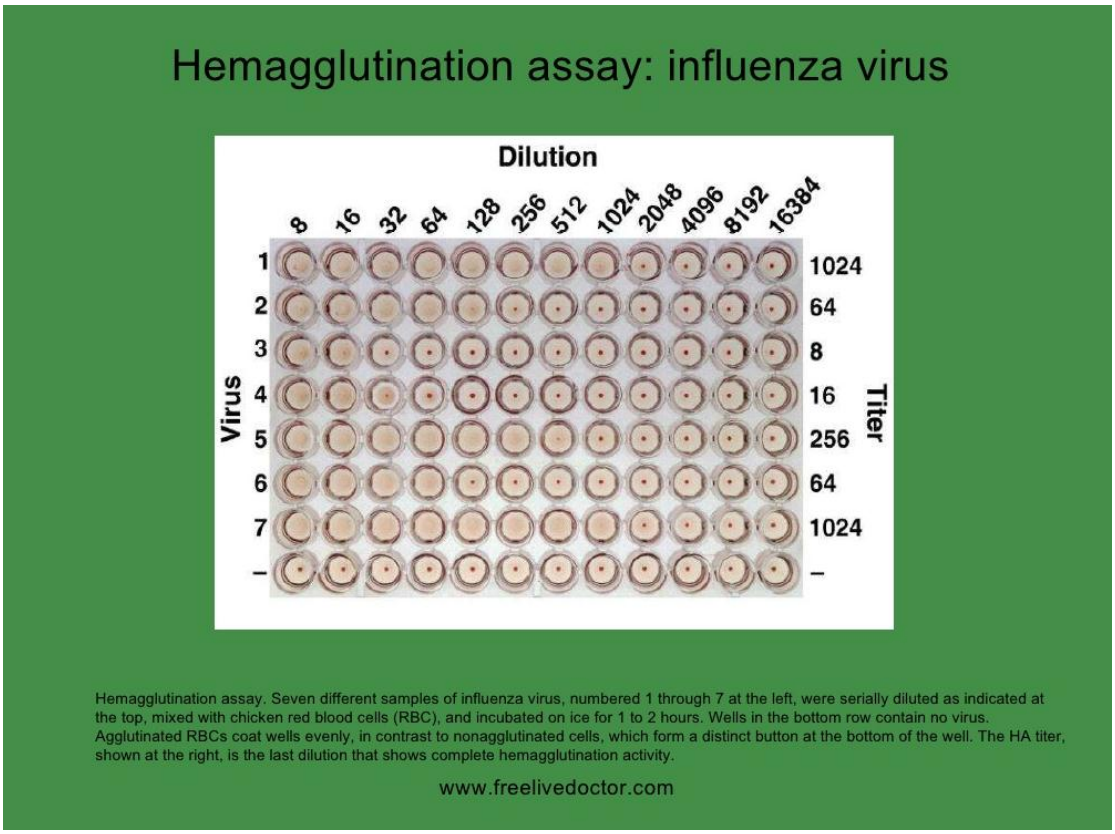
Standardization of the HI test

- It is important that there is correlation between the results of tests carried out by different technicians and in different laboratories. For this reason HI tests should be standardized both within a laboratory and between laboratories.
- Standardization is achieved by following a standard protocol. This will include:
  - Using a standard 4 HA units of the virus.
  - Using standard positive anti-serum and negative serum.
  - Including a serum control for each test serum to detect the presence of non-specific agglutinins.
  - Using a standard 1 percent dilution of red blood cells.



- **Figure 1.** An example of a haemagglutination where the positive result has failed. In the diagrammatic representation above, the haemagglutination reaction cannot be accepted. The VBS serum negative control displays a positive haemagglutination reaction as evidenced by the sheet formation at the base of the well. This indicates that an error has occurred and the results cannot be accepted. In this case, the process should be repeated.


Hemagglutination test: method
1:8
1:2
1:2
1:2
1:2
1:2
virus
serial dilution
8
16
32
64
128
256
mix with red blood cells
side view
top view
Titer = 32 HA units/ml
www.freelivedoctor.com


Hemagglutination assay: influenza virus
Dilution
8
16
32
64
128
256
512
1024
2048
4096
8192
16384
Virus
1
2
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4
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Titer
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8
16
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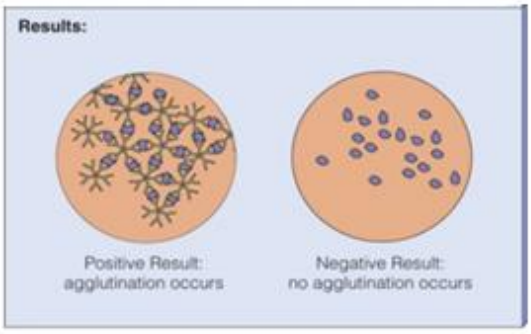
Hemagglutination assay. Seven different samples of influenza virus, numbered 1 through 7 at the left, were serially diluted as indicated at the top, mixed with chicken red blood cells (RBC), and incubated on ice for 1 to 2 hours. Wells in the bottom row contain no virus. Agglutinated RBCs coat wells evenly, in contrast to nonagglutinated cells, which form a distinct button at the bottom of the well. The HA titer, shown at the right, is the last dilution that shows complete hemagglutination activity.
www.freelivedoctor.com

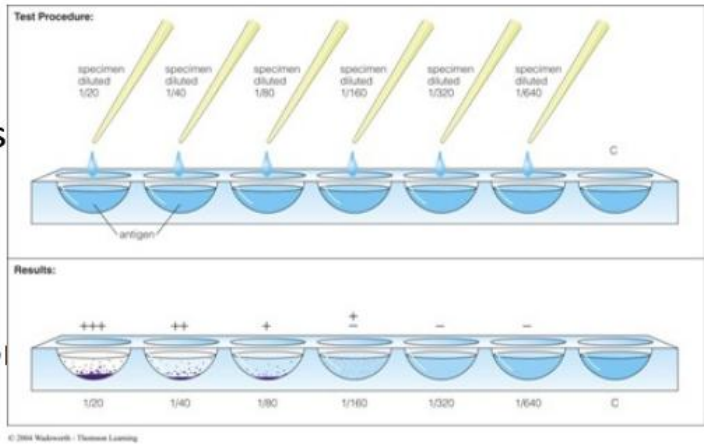
Agglutination reactions

- Agglutination
  - positive reaction
- No agglutination
  - negative reaction



Quantitative agglutination

- Similar to qualitative
- Diluted samples of antibody
- Measure amount of agglutination for each dilution



LAB-4

HAEMAGGLUTINATION ASSAY - VIRAL QUANTITATION

Haemagglutination assay was developed by American virologist George Hirst in 1941-1942. The ability of certain viruses to bind with the red blood cells through their superficial glycoproteins and proteins had been utilised to quantitate these viruses and the assay is termed as haemagglutination assay. Red blood cells when suspended with adequate amount of such viral load is capable of forming lattices coating the container. But with insufficient viral load, red blood cells form sharp dots at the centre of the container indicating absence of agglutination.

Considering these facts, the unknown samples are diluted and analysed for the last dilution point, also called end point, capable of agglutinating the red cells completely. The amount of virus in the end point is termed as one hemagglutination unit (1 HAU). Considering the following protocol, 1 HAU is the amount of virus in 50 ul volume of sample required to agglutinate 50 ul of the 0.5% chicken RBC. Additionally, the reciprocal of the dilution of the virus at end point is said to be the haemagglutination titre (HA titre).

AIM

The main purpose of the assay is to estimate HA titre for the unknown viral sample. The basis of this assay is the ability of viral haemaglutinin to bind with the sialic acid present on the receptors of surface of the red blood cells causing haemagglutination

METHODS

• Label the first row of 96-well V-bottom plate as 1- 12.

• Add 50 ul of the PBS buffer from well 2 till well 12.

• Add 100 ul of the pre-diluted virus in well 1.

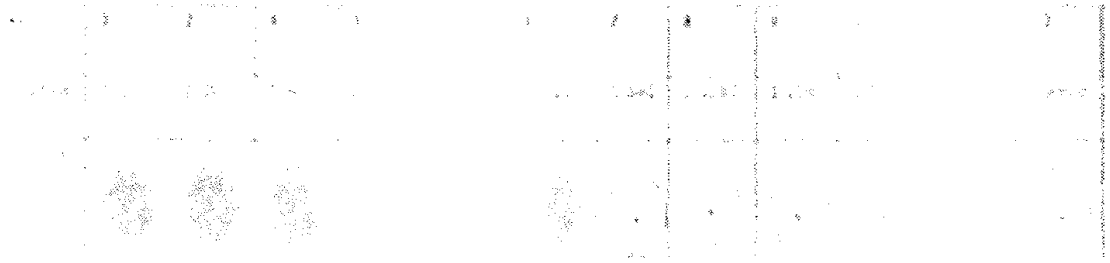
Perform serial dilution of the viral sample from well 1 to 11 using 50 ul pipette. Discard 50 ul of the diluted solution from well 11 to make the volume of the solution even in all the wells (which is required for getting the right amount of virus

in the well). Then, add 50 ul of properly mixed 0.5% chicken red blood cells to each well (1-12).

Incubate for approximately 30 minutes at room temperature (to ensure the occurrence of haemagglutination). Lastly, note the end point of the sample and observe the pattern of haemagglutination to calculate HA titre.

SAMPLE RESULT

Table: Haemagglutination p**attern of se**rially diluted influ**enza virus** for determination of HA titre .



1. Lattice formation upto well 6 shows the presence of one hemagglutination unit (1 HAU) viral load in the well.
2. HA Titre is the reciprocal of the highest dilution upto which haemagglutination was observed Here, HA titre = 320
3. Absence of hemagglutination from well 7 to well 11 - showing no or insufficient viral load to cause agglutination.
4. Well 12: Test control

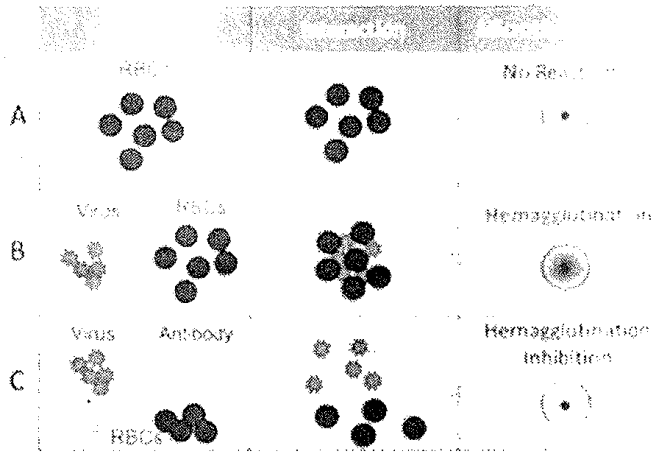
IMPORTANT NOTES

This haemagglutination assay is found to be effective, simple and easier to understand as compared to the other robust techniques, like nucleic acid amplification tests, used to assess the virus qualitatively as well as quantitatively. However, from this assay it is difficult to predict the subtypes of virus. Furthermore, if an unknown sample is to be analysed, this assay is not suitable for unravelling riddles of diagnostic approach because of the binding of red blood cells to various viruses or viral proteins. For these reasons, it is more likely to be a

screening test rather than diagnostic assay. But the viral quantitation for a known type can be performed using this assay. Despite the simpler handling, high sensitivity, lower chances of error during performance, minimal skill requirement, relatively low cost and rapid assessment, this very low specificity. Meanwhile, slight modification of the **assay to** haemagglutination inhibition assay, which detects specific antibodies against viral antigens, is much more specific that may be helpful in determining the quality of the sample effectively. In addition to that, coating erythrocytes with specific antibodies can be beneficial in increasing the specificity of the assay. Thus, evidences support the modification of haemagglutination to be useful for ascertaining both quality and quantity of viruse .

Hemagglutination Inhibition Test (HAI): Principle, procedure, result and interpretations

The nucleic acids of various viruses encode surface proteins that agglutinate the red blood cells (RBC) of a variety of species. For example; Influenza virus particles have an envelope protein called the hemagglutinin, or HA, which binds to erythrocytes, causing the formation of a lattice. This property is called hemagglutination. Reaction of viral hemagglutinins with red blood cells results in a lattice of agglutinated cells which settle irregularly in a tube or microtiter well. Unagglutinated cells settle in a compact button.



Hemagglutination and Hemagglutination Inhibition Test

Hemagglutination phenomenon is almost commonly used for diagnosis of infection produced by Orthomyxo viruses, paramyxoviruses, and the abroviruses togaviruses (including rubella), flaviviruses, and bunya viruses.

The presence of virus in infected cell cultures can be etected by hemagglutination;

the identity of the virus or of antibodies in a patient's serum can be determined by specific inhibition of that hemagglutination. Although influenza viruses can be detected by hemadsorption test, typing of the isolate is done most efficiently by hemagglutination inhibition (HAI). Reagents and conditions for the test vary by virus.