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Website:

Lecture title: **hemagglutination-inhibition-assay**

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Summary:

1- **Learning the haemagglutination inhibition test**

Hemagglutination, or **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

Principle

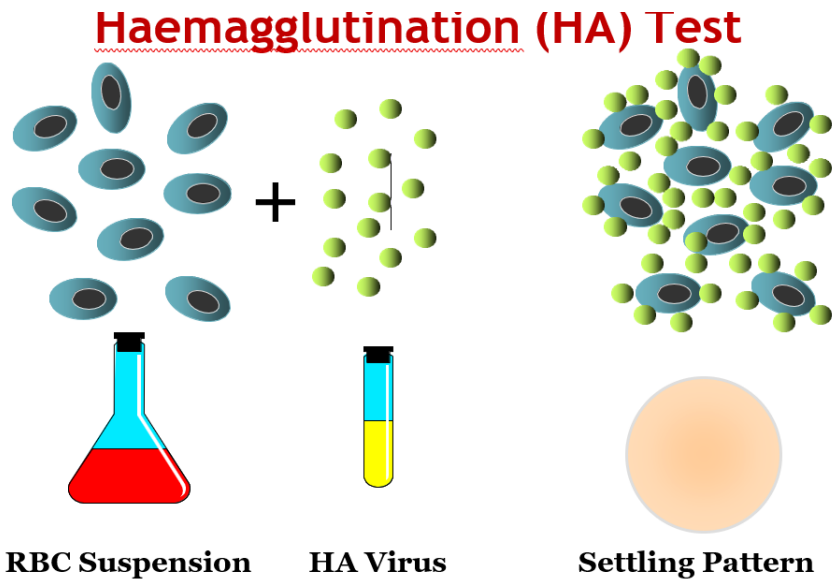
1.

Influenza virus particles have an envelope protein called the hemagglutinin, or HA, which binds to sialic acid receptors on cells.

2.The virus will also bind to erythrocytes (red blood cells), causing the formation of a lattice.

3.This property is called haemagglutination and is the basis of a rapid assay to determine levels of influenza virus present in a sample.

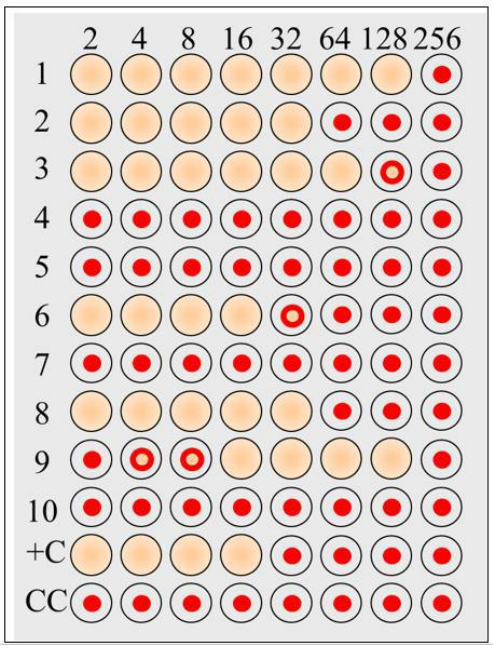
4.To conduct the assay, two-fold serial dilutions of a virus are prepared, mixed with a specific amount of red blood cells, and added to the wells of a plastic tray.



HA Procedure

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

Results

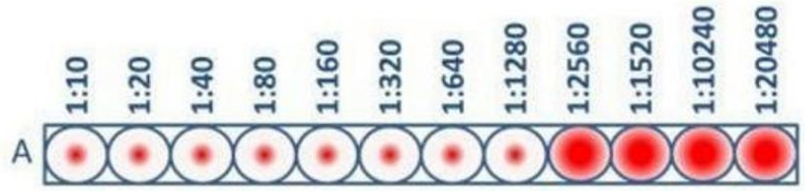


Interpretation

- 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.

Interpretation

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



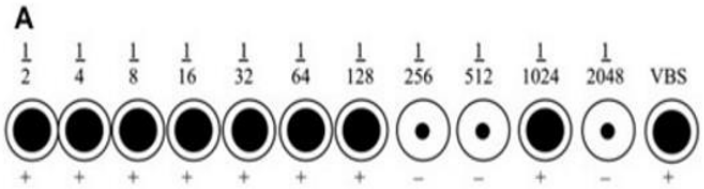
This virus sample has an HAI titer of 1280, which means that the greatest dilution of antibody that still blocked hemagglutination from occurring was at a 1280 dilution. At this dilution, the antibodies were still capable of recognising and binding to the antigens on the virus.

Quality Control

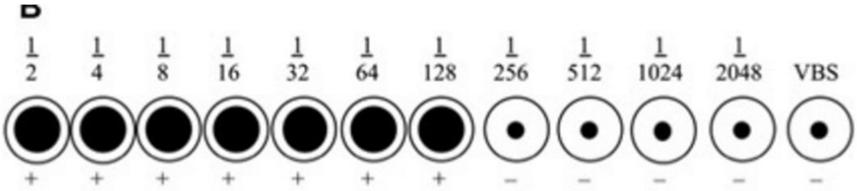
1.
Known negative serum
2.
Serum and cells without antigen (to detect nonspecific agglutination)
3.
Back titration of haemagglutination activity of the antigen (to ensure that 4 hemagglutinating virus (HAU) were tested)

Standardization of the HI test

Standardization is achieved by following standard protocol. This will include:
i.
Using a standard 4 HA units of the virus.
ii.
Using standard positive anti-serum and negative serum.
iii.
Including a serum control for each test serum to detect the presence of non-specific agglutinins.
iv.
Using a standard 1 percent dilution of red blood cells.



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.



An example of a haemagglutination where the positive result has worked correctly. In the diagrammatic representation, the heamagglutination reaction is valid. The VBS negative control displays a negative haemagglutination reaction as evidenced by the pellet that has formed at the base of the well. The last positive well is which indicates that the titre for this serum sample is 128 (i.e the reciprocal of 1/128).