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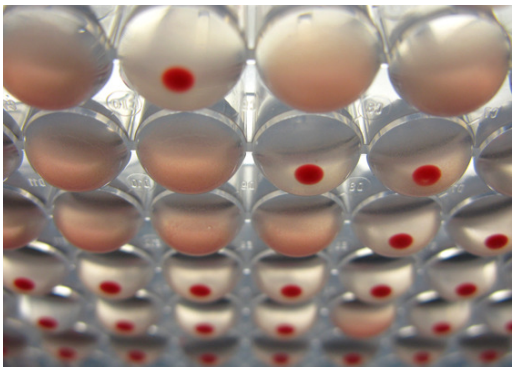
Hemagglutination (HA) Assay Protocol

The hemagglutination assay is a method for titering influenza viruses based on their ability to attach to molecules present on the surface of red blood cells. A viral suspension may agglutinate the red blood cells, thus preventing them from settling out of suspension. By serially diluting a virus in a 96-well plate and adding a consistent amount of red blood cells, an estimation of the amount of virus present can be made.

Equipment and Materials Required



- Certified Biological Safety Cabinet
- Tabletop centrifuge with appropriate fittings
- Inverted microscope (optional)
- 15 ml conical tubes
- Disposable pipettes – 1 ml, 5 ml, 10 ml
- Micropipette and sterile disposable aerosol resistant tips – 160 µl
- PBS
- Turkey red blood cells in Alsevers solution purchased from a supplier such as Lampire Biological Products
- Round-bottomed 96-well dish

Turkey RBC preparation:

1. 4 ml of turkey blood is pipetted into a 15 ml conical and topped off with PBS.
2. Spin in tabletop centrifuge at 800 rpm for 10 minutes.
3. Aspirate the supernatant without disturbing the blood cells.
4. Add 12 ml PBS and mix by inverting – do not vortex.
5. Spin at 800 rpm for 5 minutes and repeat wash two more times.
6. Aspirate supernatant after final wash and add enough PBS to make a 10% solution of red blood cells. This solution is useable for one week.
7. Make a final working solution of 0.5% RBCs in PBS.

Viral Dilution and Assay:

1. A round-bottomed 96-well dish is preferred for this assay. Flat-bottomed plates will also work, but need to be placed at an incline to develop.
2. To each well, add 50 µl PBS.
3. In the first column, add 50 µl of virus sample.
4. Mix each well and transfer 50 µl to the next well on its right. Repeat mixing and transferring 50 µl down the length of the plate. Discard 50 µl from the last well into a bleach solution.
5. Add 50 µl of 0.5% red blood cell working solution to each well. Mix gently.
6. Leave at room temperature for 30-60 minutes to develop. Negative results will appear as dots in the center of round-bottomed plates. Positive results will form a uniform reddish color across the well.
7. The virus's HA titer is a simple number of the highest dilution factor that produced a positive reading.