Detection by haemagglutination inhibition (HI)

Principle

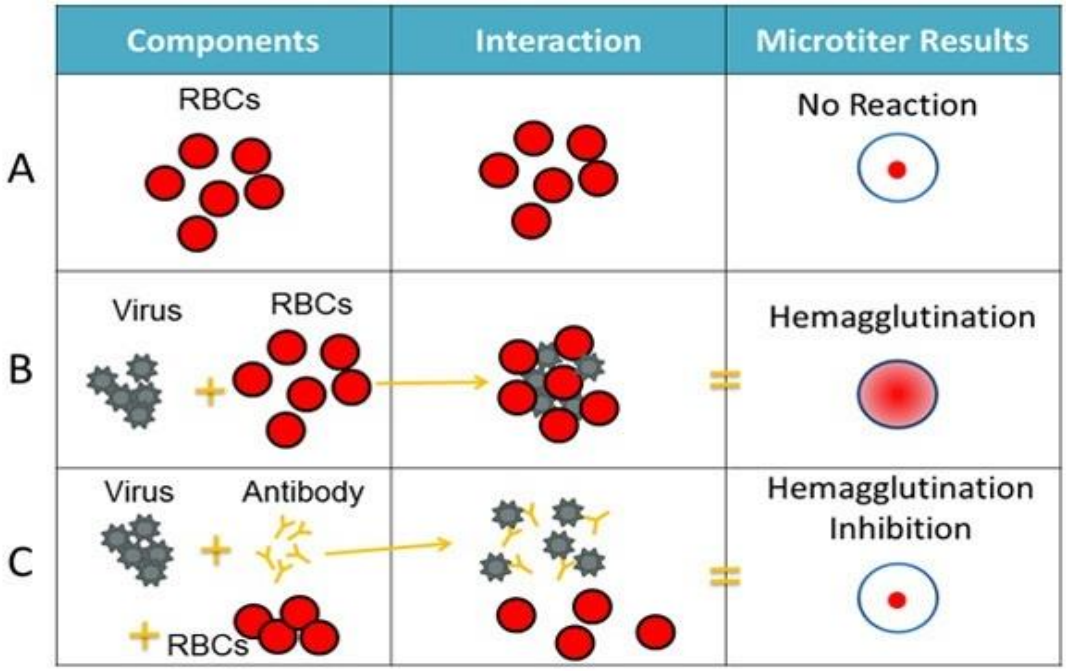
The nucleic acids of various viruses encode surface proteins that agglutinate the red blood cells (RBC) of a variety of species. For example; Influenza virus particles have an envelope protein called the hemagglutinin, or HA, which binds to erythrocytes , causing the formation of a lattice. This property 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 Orthomyxoviruses, paramyxoviruses, and the abroviruses-togaviruses (including rubella), flaviviruses, and bunyaviruses.

The presence of virus in infected cell cultures can be detected 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.

The basis of the HAI assay is that antibodies to that particular virus (for example- influenza virus) will prevent attachment of the virus to RBC. Therefore hemagglutination is inhibited when antibodies are present.



HAI Titer: The highest dilution of serum (Ab) that prevents hemagglutination is called the HAI titer of the serum.

1. If the serum contains no antibodies that react with influenza virus, then hemagglutination will be observed in all wells.
2. Likewise, if antibodies to the virus are present, hemagglutination will not be observed until the antibodies are sufficiently diluted.

The HAI test may be complicated by the presence of non-specific inhibitors of viral haemagglutination and naturally occurring agglutinins of the erthrocytes. Therefore, the sera should be treated before use or false positive or negative results may arise.

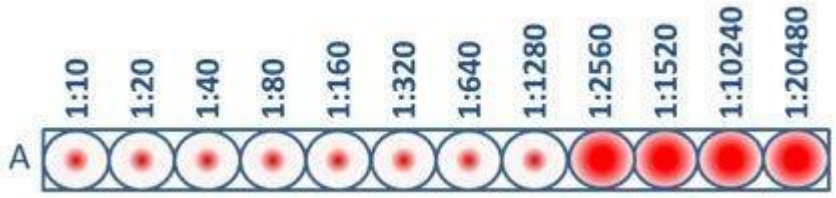
Materials and Reagents:

1. Red cells from an appropriate species (Chicken, goose, guinea pig, trypsinized human O) collected in Alsever's solution or heparin
2. Diluent (e.g. Bovine albumin veronal buffer) at appropriate pH
3. Solutions to remove nonspecific hemagglutinins from serum
4. Infected cultural fluid or standard antigen (e.g preparation of influenza virus) for serology

Procedure

Obtain a preparation of virus (e.g. influenza viruses) with known HA titer or determine its HA titer

1. Prepare two-fold dilutions of patient/test serum to be tested e.g. from 1:4 to 1:1024.
2. Add a fixed amount of virus to every well of a 96-well plate, equivalent to 4 HA units (varies according to virus), except for the serum control wells.
3. The plate is then allowed to stand at room temperature for 60 minutes (time varies according to specific requirements).
4. Add (0.5%) red blood cells (RBC) and incubate at 4^{o}C for 30 minutes.
5. Read the wells.



Results/interpretation

The highest dilution of serum (Ab) that prevents hemagglutination is called the HAI titer of the serum.. A smooth or jagged shield of cells or an irregular button indicates agglutination. Observation of movement of the button of red cells when the plate is tilted may help to clarify the end point.

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 1280 dilution. At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus.

The advantages of HAI tests are

- ❖ They are relatively easy
- ❖ Inexpensive to perform.

The disadvantages are that HAI tests are

- ❖ Not as sensitive as EIAs or RIAs,
- ❖ The actual reading of results is subjective the reagents should be fresh or else abnormal agglutination patterns may arise which makes the reading and interpretation of the test very difficult. As a result the HAI test for rubella had been replaced by more sensitive and reliable EIA and RIA tests for rubella IgG in many virus diagnostic laboratory

Note: non-specific inhibition of haemagglutination in sera can be inactivated by

1. Heating at 56 ºC for 30 min
2. Treatment with kaolin, trypsin periodate
3. Bacterial neuraminidase