

HI

0.025 ml of PBS is dispensed into each well of a plastic V-bottomed microtitre plate. ii) 0.025 ml of serum is placed into the first well of the plate. iii) Twofold dilutions of 0.025 ml volumes of the serum are made across the plate. iv) 4 HAU virus/antigen in 0.025 ml is added to each well and the plate is left for a minimum of 30 minutes at room temperature, i.e. about 20°C, or 60 minutes at 4°C. v) 0.025 ml of 1% (v/v) chicken RBCs is added to each well and, after gentle mixing, the RBCs are allowed to settle for about 40 minutes at room temperature, i.e. about 20°C, or for about 60 minutes at 4°C if ambient temperatures are high, when control RBCs should be settled to a distinct button. vi) The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/antigen and PBS controls) should be considered to show inhibition. vii) The validity of results should be assessed against a negative control serum, which should not give a titre >1/4 (>22 or >log2 2 when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre. The value of serology in diagnosis is clearly related to the expected immune status of the affected birds. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (24 or log2 4 when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 (23 or log2 3) or more. Back titration of antigen should be included in all tests to verify the number of HAU used. In vaccinated flocks that are being monitored serologically, it may be possible to identify anamnestic responses as the result of a challenge infection with field virus (Alexander & Allan, 1974), but great care should be exercised as variations may occur from other causes. For example, it has been demonstrated that APMV-3 virus infections of ND-virus-vaccinated turkeys will result in substantially increased titres to NDV

**HEMAGGLUTINATION-INHIBITION TEST (HI)**

Purpose: To quantitate serum antibody to a specific avian (antigen)

1.  Avian viruses which can agglutinate avian RBCs (hemagglutination) include NDV, Influenza, and Adeno127. In addition to viruses, Mycoplasmas are also capable of hemagglutinating avian RBCs.

2. Antibodies directed against these organisms will inhibit hemagglutination.

**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) 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

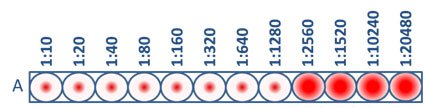
**Procedure**

Obtain a preparation of virus (e.g. ND 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 red blood cells (RBC) and incubate at 4 ºC 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

[](http://i2.wp.com/microbeonline.com/wp-content/uploads/2014/12/hi-titer-assay.jpg)

*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.*