Influenza A Virus: Nucleoprotein (NP) Antibody Inhibition Test

*** Please note MODIFIED protocol as of February 2013***

Product Insert

Cat. No. IAV207-5 (5 x 96 wells)

For Research Use Only

Introduction

Influenza viruses can be divided into three classes, A, B, and C, largely based upon conserved antigenic differences in the internal nucleoprotein. Influenza A virus, typically encountered more frequently than types B and C, and associated with the majority of serious epidemics, can be further subdivided into strains or subtypes based on antigenic differences in the external hemagglutinin proteins (H1-H16) and neuraminidase proteins (N1-N9).

A variety of wild waterfowl appear to be the predominant natural reservoir for Influenza A viruses and subtypes representing most of the hemagglutinin and neuraminidase combinations can be found circulating in these birds. Historically, human influenza virus infections have been associated with H1N1, H2N2, and H3N2 subtypes of influenza A, although a recent (1997) and significant outbreak in Hong Kong was identified as an H5N1 subtype. This outbreak was not only significant because it resulted in 18 human infections and 6 deaths, but it also represented the first known demonstration of avian influenza virus transmission to humans.

Depending upon the serological requirements or research interest (natural infection, vaccine monitoring, or DIVA [Differentiation of Infected from Vaccinated Animals]), it may be useful to monitor the development of influenza A NP-specific antibody in a variety of species.

Virusys Corporation

P.O. Box 56 ● Taneytown, MD 21787
Website www.virusys.com E-mail support@virusys.com

Virusys has developed a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the detection of influenza A NP-specific antibodies in serum from human and veterinary sources. Because the assay utilizes "inhibition of binding" technology, it may be used with serum from any species, and therefore does not require species-specific conjugates. The assay can be completed in less than 1.5 hr., contains only one wash step, and incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from sample matrix and/or the nonspecific adsorption of reactive test components. The result is an assay that is both highly sensitive and specific (see Performance Section).

Components

1. IAV Antigen Capture Plate (96 tests) - 5 ea.
2. IAB Antigen Diluent (1x) - 60 ml
3. IAB Antigen Concentrate (1x) - 10 ml
4. IAB Positive Serum Control (2x) - 0.1 ml
5. IAB Negative Serum Control (2x) - 0.1 ml
6. Wash Buffer (20x) - 2 x 50 ml
7. IAB Detection Antibody, HRP-labeled (1x) - 30 ml
8. Chromagen Solution (1x) - 60 ml
9. Stop Solution (1x) - 60 ml
10. Sample Dilution Tray - 5 ea.

Storage

Store all kit components at 2-8° C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

Procedure

1. Remove the kit components from storage and allow to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample. In addition, include two wells each for the **Antigen Diluent Control**, **Positive Serum Control** and the **Negative Serum Control**.
3. Based upon the number of wells required for the assay, prepare **Sample Diluent** from the **IAB Antigen Concentrate** and **IAB Antigen Diluent** using a ratio of 1 ml of **Antigen Concentrate** to 5 ml **Antigen Diluent.** For example, if using the entire plate combine 2 ml of **Antigen Concentrate** with 10 ml of **Antigen Diluent.**
4. To begin the assay, transfer 5 µl of each serum control (two wells each) and each sample (one well each) to the appropriate wells of the dilution tray.
5. Add 100 µl of **IAB Sample Diluent** to each sample, positive control, and negative control well. Mix by pipetting up and down several times.

6. Add 100 µl of the **Antigen** Diluent to the appropriate wells (NOTE: Wells containing **Antigen** Diluent DO NOT receive Sample Diluent).
7. Incubate for 10 min. at room temperature.
8. Transfer 50 µl of sample or control to the appropriate wells of the **IAB Antigen Capture Plate**.
9. Cover the plate and incubate for 30 min. at room temperature on a plate shaker set at moderate speed.
10. Add 50 µl of **1x IAB Detection Antibody** to each well. DO NOT WASH THE PLATE AT THIS TIME. Cover the plate and incubate for 30 min. on a plate shaker using the same settings (Step 6).
11. Wash the wells 6x with **1x IAB Wash Buffer**.
12. Add 100 µl of **IAB Chromagen** to each well and incubate for 10 min. on a plate shaker.
13. Stop the reaction by the addition of 100 µl of **IAB Stop Solution**.
14. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

Quality Control

1. The negative serum control absorbance values should be ≥0.600
2. The positive serum control absorbance values should be ≤0.300.
3. The diluent control absorbance values should be ≤0.300.
4. The mean absorbance value for the positive control serum should be ± 15% of the mean absorbance value for the diluent control.

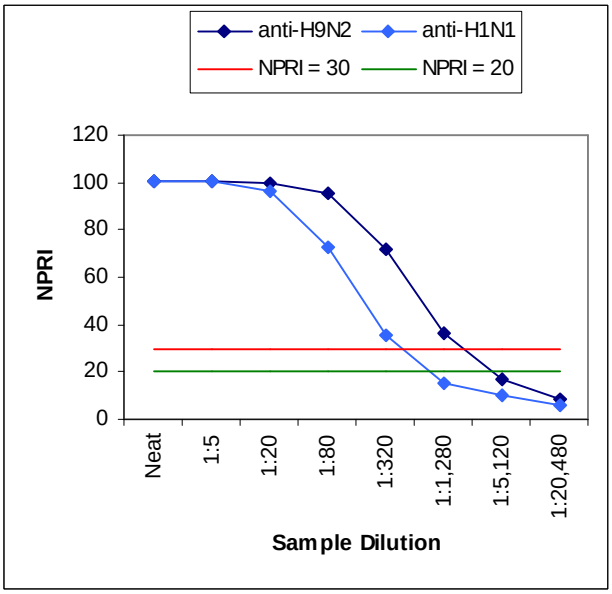
Determination of NP Reduction Index and Interpretation of Results

A positive result with the Virusys Influenza A Nucleoprotein Antibody Inhibition Test is dependent upon the presence of NP-specific antibody in the sample. Binding of the antibody in the sample to NP antigen in the sample diluent will result in the complete or partial inhibition of NP binding to the antibody coated on the IAB Antigen Capture Plate. The resulting decrease in NP binding can then be used to calculate a **NP Reduction Index** for each sample.

1. To calculate the NPRI for each sample, it is first necessary to calculate the mean absorbance values for the diluent control and the positive and negative controls.
2. Subtract the mean value of the diluent control from the mean of the positive serum control (maximal NP removal) and from the mean of the negative serum control (minimal NP removal) and the absorbance values for all samples.
3. Calculate the NPRI using the following formula: **NPRI = (1-[Delta Sample Abs/Delta NC Abs]) x 100**
4. A NPRI >30 is indicative of the presence of anti-influenza A NP antibody.
5. A NPRI <20 is considered negative for the presence of anti-influenza A NP antibody.
6. A NPRI ≥20 but ≤30 represents an equivocal result, suggestive of the presence of NP antibody. Another sample should be obtained in 7-14 days and tested (preferably with the original equivocal sample).

Performance

Sensitivity: Titration of anti-H9N2 and anti-H1N1 positive chicken sera. (NOTE: sample dilutions represent dilution of positive sera in pool of normal [negative] chicken serum. Each dilution was then diluted 1:21 into IAB Sample Diluent for assay as described above).



Specificity: NPRI values for hyperimmune sera obtained from SPF chickens immunized with the following viruses (all NPRI<10):

Infectious Bronchitis Virus (Ark):	0
Infectious Bursal Disease Virus:	7
Infectious Laryngotracheitis Virus:	7
Newcastle Disease Virus:	4
Marek's Disease Virus:	4
Avian Adenovirus (Type 1):	1

Virusys Corporation
P.O. Box 56 ● Taneytown, MD 21787
Website www.virusys.com E-mail support@virusys.com