Saint Luke's Regional Laboratories
Clinical Laboratory Letter
February 2012

What Does Influenza H#N# Really Mean?

Major outbreaks of influenza are associated with influenza virus type A or B. Influenza A is responsible for frequent, usually annual outbreaks or epidemics of varying intensity, and occasional pandemics, whereas influenza B causes outbreaks every two to four years. The main reason that influenza B viruses do not cause pandemics is that they primarily infect humans and seldom infect animals. Nearly all adults have been infected with influenza C virus, which causes mild upper respiratory tract illness.

Influenza viruses are classified using the following information:

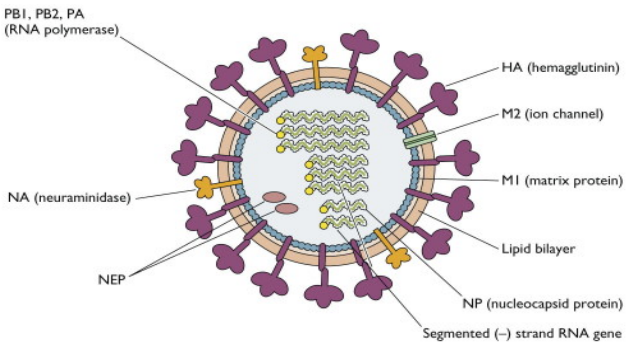
- Type A, B or C/place isolated/number of isolate/year isolated
- Influenza A is divided into subtypes according to their hemagglutinin (H) and neuramidase (N) proteins.

Examples of influenza virus names are A/California/7/2009 (H1N1) and B/Brisbane/60/2008.

The influenza virus is an enveloped virus, meaning that the outer layer is a lipid membrane which the virus acquires from the host cell. Inserted into the lipid membrane are the viral glycoproteins, hemagglutinin (H) and neuraminidase (N). Influenza A virions have three membrane proteins (H, N and M2), while Influenza B virions have four (H, N, NB and BM2). Beneath the lipid membrane is the M1 viral matrix protein that provides strength and rigidity to the viral envelope. M2 protein is a proton channel that is the target of the antiviral drugs amantadine and rimantadine. Within the influenza A or B virion are eight segments of viral RNA that carry the all the genetic information needed to synthesize new virus particles.

Antigens on the internal proteins M1 and NP are type-specific and used to determine if a particular influenza virus is type A, B or C. Both M1and NP proteins of all members of each type exhibit cross reactivity.

Hemagglutinin is a surface glycoprotein that binds to sialic acid residues on respiratory epithelial cell surface glycoproteins. This interaction is necessary for attachment and fusion of viral and epithelial cell membranes. Neuraminidase digests sialic acid (neuraminic acid) on the surface of target cells, promoting entry of the virus into the cell. Neuraminidase also facilitates penetration of the mucus layer in the respiratory tract. By late infection, almost all sialic acid has been removed from infected cell surface making it is easier for progeny virions to disseminate to other cells. N is the target of the antiviral drugs Relenza and Tamiflu.



H and N exhibit more antigenic variation than the internal proteins and are the major determinants of Influenza A subtype and strain-specificity. There are 16 H and 9 N variants, but each virus has only one H and one N variant.

Minor changes in the envelope glycoproteins, hemagglutinin and neuraminidase, are referred to as antigenic drift, and major changes are called antigenic shifts. Antigenic drifts are associated with localized outbreaks, while antigenic shifts are associated with epidemics and pandemics of influenza A.

Antigenic drift is due to a point mutation in HA and/or NA. Inefficient proofreading by influenza viral RNA polymerase results in a high incidence of transcription errors and amino acid substitutions in hemagglutinin or neuraminidase, allowing new variants to evade preexisting humoral immunity and cause influenza outbreaks. An individual immune to the original strain is not immune to the drifted one.

Antigenic shift is due to HA or NA gene reassortment that results in synthesis of new H and/or N protein variants. Wild aquatic birds are the

natural hosts for all subtypes of influenza A virus. Pigs also play an important role in the evolution of human pandemic strains because pig trachea contains receptors for both avian and human influenza viruses and pigs support the growth of both types of viruses. Genetic reassortment between avian and human virus may occur in pigs, leading to novel strains.

When a pig becomes infected with both human and avian viruses, the RNAs of both viruses are copied in the nucleus. When new virus particles are assembled at the cell membrane, some of the RNA segments may originate from either of the infecting virus. New viruses that inherit RNA from both avian and human influenza are called reassortants. They may contain human internal proteins and animal H and/or N proteins. If this virus reassortant can infect humans, they will have little immunity to it, increasing the likelihood of an epidemic or pandemic. The H1N1 pandemic that occurred in 2009 was due to reassortment of avian, human and swine influenza viruses.

Reassortment can only occur between influenza viruses of the same type. It is not understood why influenza A viruses never exchange RNA segments with influenza B or C viruses. Influenza B is much less likely to undergo antigenic shift because there is not an animal reservoir for this virus.

Although 16 H and nine N virus subtypes occur in their natural reservoir of aquatic birds, only three hemagglutinin subtypes (H1, H2, and H3) and two neuraminidase subtypes (N1 and N2) have established stable lineages in humans and caused widespread human respiratory infection. H1N1 and H3N2 cause most of the seasonal epidemics today.

Ketone Testing Change

Detection of ketones in urine and blood is used in the management of diabetes mellitus. American Diabetes Association criteria for diabetic keotacidosis include plasma glucose ≥250 mg/dL, serum anion gap >10 mEq/L, bicarbonate ≤18 mEq/L, blood pH≤7.30 and ketosis. Other conditions that can produce elevated levels of ketones include malnutrition, starvation, alcoholism, and some inborn errors of metabolism.

Ketone bodies are catabolic products of free fatty acids and are normally present in urine and blood at very low concentrations. The two major mechanisms for increased ketone levels in patients with diabetes are increased production from triglycerides and decreased utilization by the liver, both due to relative or absolute insulin deficiency.

The three ketone bodies are acetoacetate (AcAc), acetone, and beta-hydroxybutyric acid (BHB). Normally, AcAc and BHB are present in approximately equimolar amounts. Acetone is derived from the breakdown of AcAc and is present in much lower concentration. In all of the disease states associated with ketosis, the equilibrium between AcAc and BHB is shifted toward BHB formation. The typical diabetic patient with ketoacidosis usually has 78% beta-hyroxybutyrate, 20% acetoacetate, and 2% acetone.

For years, ketoacidosis was diagnosed and monitored with a nitroprusside-based test, commonly known as the Acetest. This test measures only AcAc and to a lesser extent acetone but does not detect BHB. Thus, Acetest is relatively insensitive for detecting the early stages of ketoacidosis.

Recently, Acetest has become indefinitely backordered due to manufacturing problems. Because of the short supply and insensitivity of Acetest, Saint Luke's Regional Laboratories has decided to discontinue Acetest and begin offering BHB. This test will be phased into SLHS laboratories during the next month. It will be orderable as beta-hydroxybutyrate. The test is performed on a drop of whole blood obtained by fingerstick. Reference range is <0.6 mMol/L.

Glucose Reference Range Change

To comply with the American Diabetes Association guidelines, the reference range for plasma glucose was changed to 70-100 from 65-100 mg/dL.