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Review Article

Influenza Virus a Respiratory Tract Infection to Human and a Zoonosis Virus Infecting Animals and Birds

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Received: July 29, 2020; **Published:** August 11, 2020

Abstract

Influenza viruses are respiratory tract infection disease know by the name 'flu' and are belong to the virus family Orthomyxoviridae. They are zoonosis infectious agent infect human and vertebrates. There are four common species of influenza viruses. These are types A, B, C and D. Type A virus is the most virulent infection to human divided into subtypes based on two spike glycoproteins expressed on the virus surface. These two glycoproteins are hemagglutinin (HA) and neuraminidase (NA). There are 18 different hemagglutinin subtypes (H_1 through H_{18}) and 11 different neuraminidase subtypes (N_1 through N_{11}) for influenza A virus. Influenza B virus also infect human and rarely animals, but it is less common than influenza A virus. Influenza C virus is less common than both Influenzas A and B viruses, causing mild endemic illness to human specially children, and infect animals such as dogs and pigs. Influenza D virus infect cattle, pigs and there is no evidence yet that it can infect human. Influenza viruses A and B causes seasonal epidemics of disease mostly in winter and influenza A virus subtypes are known to cause influenza (flu) pandemics illness. These influenza viruses spread through air from coughs or sneezes of infected person. Also, can spread from person to person contact or by touching surfaces contaminated with the virus then touching eyes, nose or mouth. World Health Organization (WHO) estimates that annual influenza epidemics worldwide is in the range of 3 to 5 million with severe illness, causing 250,000 to 500,000 annual deaths.

Keywords: *Influenza Viruses' Structure; Hemagglutinin; Neuraminidase; Mechanism of Infection; Influenza Virus Drifting; Influenza Virus Shifting; Virus Diagnostics; Molecular Assay; Immunoassay; Serology Testing; Flu Vaccines; Flu Antiviral Drugs*

Introduction

Influenza viruses are belonging to Orthomyxoviridae family [1], represents enveloped viruses with antisense single-strand RNA segments (ssRNA). The virus structure (Figure 1) is a spherical or filaments in shape, with helically symmetric nucleocapsid (virion) consist of a nucleoprotein (NP) and genome segments of seven to eight single-stranded antisense RNAs. These RNAs are packaged with nucleoprotein into a helical ribonucleoprotein (RNP), with three polymerase peptides (PB1, PB2 and PA), for each RNA segment. The outer layer (envelope) is a lipid bilayer structure from the plasma membrane of infected host cell. Virus surface is spiked with a trimeric glycoprotein hemagglutinin (HA) and neuraminidase (NA) consist of four polypeptides. Hemagglutinin (HA) function is to attach the virus to the host cell protein [2]. Neuraminidase (NA) is enzyme facilitate entering and releasing newly replicated virus from the host cell [3]. Hemagglutinin (HA) and neuraminidase (NA) are antigens for influenzas A and B viruses. Both viruses undergo genetic variation [4]. The inner side of the envelope that surrounds the virion is an antigenic M1 matrix protein lining, and within is virus genome, organized into eight pieces (segments) of single-stranded RNAs for both influenza A and B viruses (Figure 2). These RNA segments are the basis for the emerging of newly strains of influenza A and B viruses. The genome for affluenza C virus is organized into, seven segments of single-stranded RNAs. Influenza C virus is antigenically stable comparing to influenza A and B viruses. All influenza viruses RNAs packaged with nucleoprotein (NP) in a helical ribonucleoprotein (RNP) structure, with three polymerase peptides for each RNA segment. These polymerase peptides

consist of three polymerase proteins PB1, PB2, and PA located at the ends of the nucleocapsids (Figure 2). These polymerase proteins are essential for the virus replication [5].

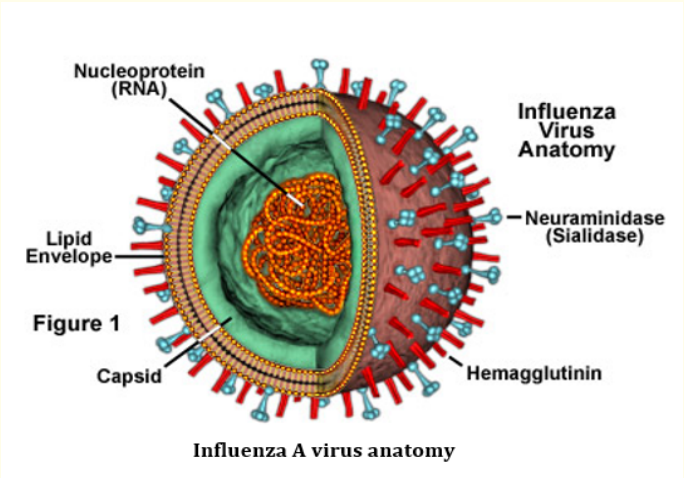

Figure 1: *The diagram show the virus structure consist of Neuraminidase (NA), Hemagglutinin (HA), Lipid layer (envelop), Capsid consist of M1 matrix protein that give the virus rigidity and contain the RNA, and Nucleoprotein (RNA).*

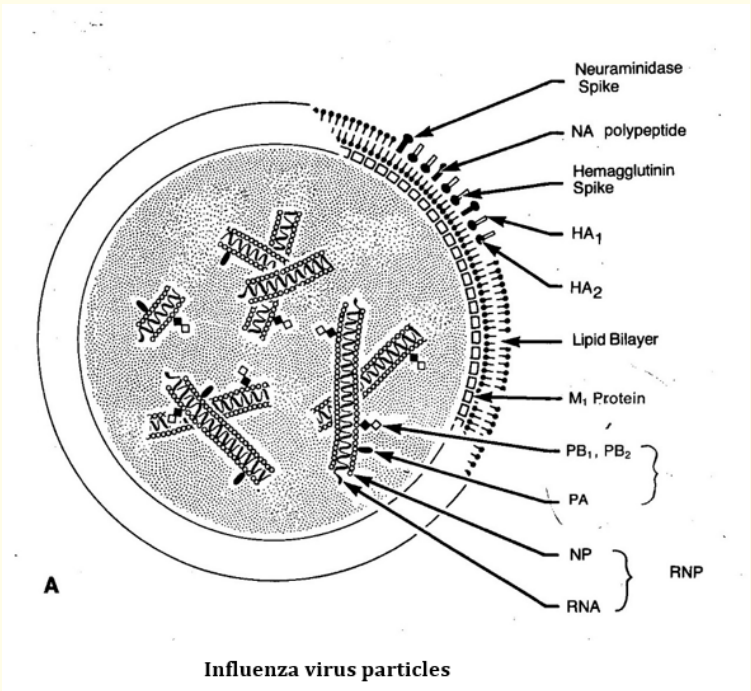

Figure 2: *This virus diagram show (1) Neuraminidase spike (NA) composed of four NA polypeptides, (2) Hemagglutinin (HA) spike composed of three sets of HA_1 and HA_2 polypeptides, (3) Virus envelope is a lipid bilayer, and matrix protein (M_1) give the virus rigidity and shape (3) Ribonucleoprotein (RNP) is RNA segments gives the appearance of double helix RNA due to the association of internal Nucleoprotein (NP) (4) PB1,PB2,and PA proteins on a single stranded of RNAs.*

Influenza virus drifting and shifting

Mutations (Figure 3) occurred in the antigenic structure of hemagglutinin (HA) and neuraminidase (NA) for both influenza A and B viruses causing a number of different influenza subtypes and variants respectively. Mutants of influenza A virus are named according to the particular antigenic determinants of surface proteins hemagglutinin (HA) into 13 major subtypes and neuraminidase (NA) into 9 major subtypes [6]. These subtypes of influenzas A viruses are named for example into A (H2N1), A (H3N2), etc. These new subtypes of influenza A viruses are emerged due to gradual process know by the name antigen drifting and antigen shifting. Antigen drifting [7] the mutation occurred within the virus antibody-binding sites of hemagglutinin (HA) and neuraminidase (NA) facilitate the new emerged virus strain to escape the host immune system for a person already built immunity to old strains of influenza A virus. Both influenza A and B viruses continually undergo antigenic drifting causing the need to continue developing new vaccines against newly emerged virus strains. Antigen shifting is another type of virus mutation only occurs for influenza A virus [8]. This antigenic shifting is caused by the virus genomic (RNAs) recombination that occurred when host cell simultaneously infected by two different strains of influenza A viruses, causing newly emerged major completely different strain of influenza A virus.

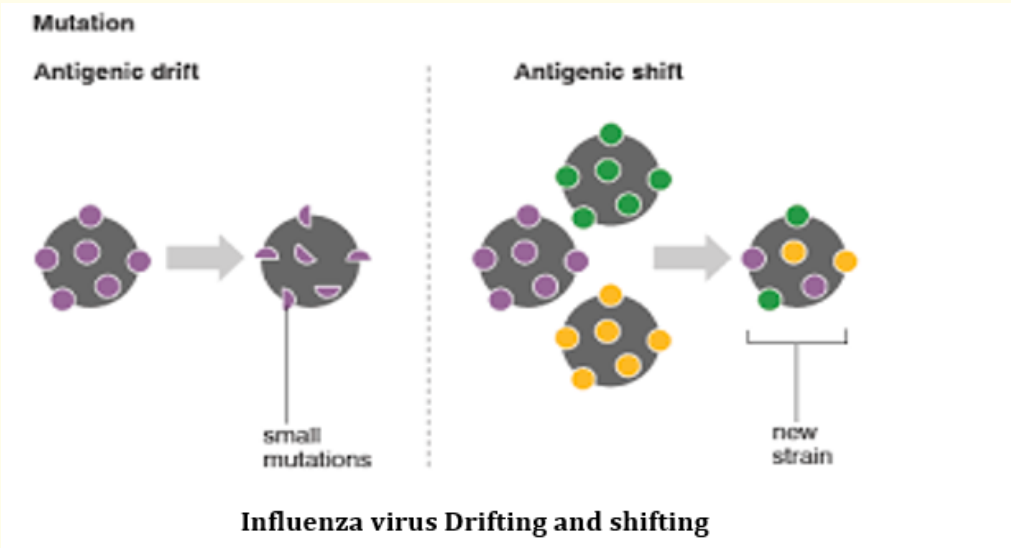

Figure 3: *Frequent antigenic change of Influenza viruses that occurs in hemagglutinin (HA) or neuraminidase (NA). These two surface antigens of influenza virus undergo antigenic variation independent of each other. These changes are Antigenic Drift a miner antigenic change, and Antigenic Shift a major antigenic change.*

Influenza virus subtypes

Influenza A virus can be subdivided into different subtypes based on the antigen structure of hemagglutinin (HA) and neuraminidase (NA) glycoproteins spiked on the virus surface. There are 16 subtypes for hemagglutinin (HA) and 9 subtypes of neuraminidase (NA), therefore there are potentially 144 (H N) different subtypes of influenza A virus, among them, is the most two commonly virulent subtypes for human. These two common virulent subtypes are H1N1 and H3N2. Hemagglutinin (HA), neuraminidase (NA) on the surface of influenza A virus genetically mutated continuously causing emerging many subtypes of influenza A virus. Continuous genetically mutation of Hemagglutinin (HA) and neuraminidase (NA) on virus's surface require continuously developing influenza (flu) vaccines for people vaccination on annual basis. Some subtypes of influenza A virus are commonly infecting human and vertebrates, these are [9], H1N1, H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3, H10N7, H7N9 and H6N1. From these subtypes are Spanish flu H1N1 [10] caused over 50 million death in 1918, Asian flu H2N2 [11] caused 2 million death in 1957, Hong Kong flu H3N2 [12] caused one million

death in 1968, and swine flu H1N1 caused over 14,000 death in 2009. Swine flu H1N1 was the same H1N1 pandemic Spanish flu subtype in the year 1918.

Influenza B virus is not divided into subtypes, but instead classified into two lineages based on the antigenic properties of its surface glycoprotein hemagglutinin (HA) into influenza B/Yamagata, and influenza B/Victoria.

Influenza virus's mechanism of infection

Influenza virus's infection and replication occurred in the host lung epithelial cell (Figure 4), hemagglutinin (HA) on the virus surface bind to its specific host cell receptor, this host cell receptor is the sialic acid of the glycoprotein that express on the surface of host cell membrane [13]. Once the virus binds to the host cell receptor it inters the cell cytoplasm via endocytosis process and released into the cell endosome [14]. In this internalization process, the virus hemagglutinin (HA) is cleaved by the virus neuraminidase (NA) enzymes into a short peptide sequence of HA known by name fusion peptide [15]. This fusion peptide interacted with the host cell membrane receptor (sialic acid) causing the virus to release into the host cell cytoplasm, and into the endosome. Inside the endosome, the virus genomic materials (RNAs) is released and entered the cell nucleus [16]. Endosome acidification caused (H^+) interring the M2 ion channel and dissociate the virus M1 matrix protein from the virus ribonucleoprotein (RNP) and releasing the virus genetic materials (RNAs) into the host cell nucleus. Once the virus RNAs is inside the host cell nucleus, it is transcribed into virus messenger RNA (mRNA), and virus mRNA is polyadenylation for viral proteins translations into ribonucleoprotein (RNP) using host cell machinery of rRNA, and tRNA [17]. Synthesized viral ribonucleoprotein (RNP) is exported from the host cell nucleus into the host cell cytoplasm for viral protein hemagglutinin (HA) and neuraminidase (NA) glycosylation in the host cell endoplasmic reticulum and transported via the host cell Golgi network. Both synthesized viral ribonucleoproteins and RNA segments are assembled into partial mature virus cells and transported to the host cell membrane, where packaged to generate mature viral particle buds that are released from infected cells via budding process to infect neighboring healthy cells in the host (patient) to continue virus replications cycles. In addition, replicated viruses are also released through the patient mouth, nose and eyes contaminating the air with infected secretion that infect healthy person via person to person contact or via the contact with dry surface contaminated with respiratory droplets.

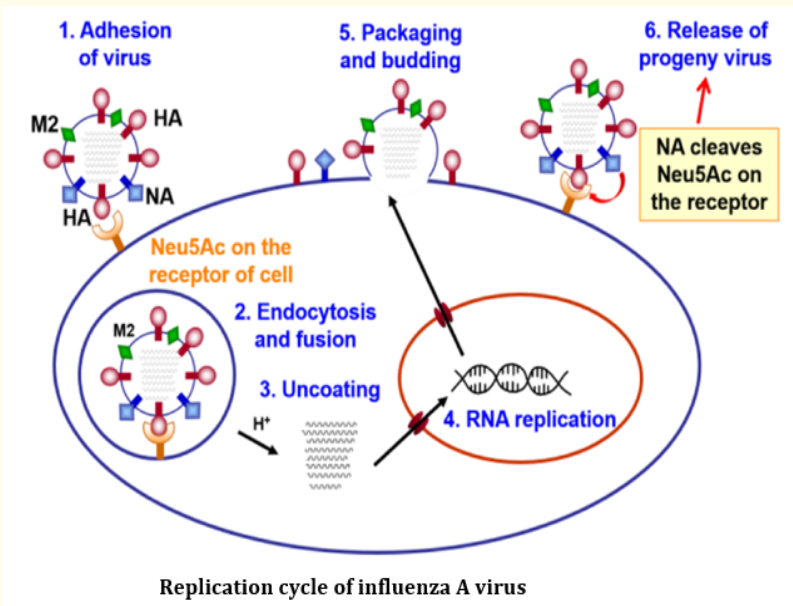

Figure 4: *Virus mechanism of infection initiated by the virus binding and entering host epithelial cells, fusion with endosomal membrane, virus uncoating, releasing viral RNA to inter the host cell nucleus, virus RNA replication and transcribe into mRNA within the nucleus, synthesis of virus structural, budding out the virus envelope, finally releasing mature viruses that are capable to infect neighboring host epithelial cells.*

Transmission and symptoms of influenzas virus's infection

People with influenza (flu) illness can spread the virus to others up to about 6 feet away, and the virus transmit mainly by droplets made when a person with influenza (flu) symptoms cough, sneeze or talk. These droplets can land in mouths or noses of people who are nearby. Also, a person might get infected by touching a surface or object that is contaminated with influenza virus droplet and then touch mouth, nose, or possibly eyes. Symptoms of virus infection can take 1 to 4 days after the virus enters the body, and the infected person can be contagious in the first three to four days after the infection [18]. Some people are asymptomatic (infected with the influenza virus and does not show flu symptoms) but may still spread the virus to others.

In summary, infected healthy person may infect others before illness symptoms developed, and up to 5 to 7 days after symptoms of illness appeared. Also, a person with weekend immunity might be able to infect others for longer than 7 days after symptoms of illness appeared. A patient with Influenza (flu) illness feels some or all of the following symptoms: fever, chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, and fatigue (tiredness). Some patients specially children might have vomiting and diarrhea in addition to these common flu symptoms.

Diagnostic tests for detection of influenza virus in respiratory specimens

Influenza (flu) illness is a seasonal endemic infection, and both influenzas' A and B viruses are the mainly agents [19]. Testing patients for Influenza virus infection is not generally necessary by physician to make clinical diagnosis specially in the seasonal circulation of influenza viruses in a local community. Influenza viruses testing are important for epidemiologists and health managements to identify influenza virus type and strain that is circulating in the community for control measure developing, and to implement the infection prevention by vaccination the community population with selected effective vaccine to stop influenza (flue) spreading [20]. In addition, patients with suspected influenza infection that are being admitted to hospitals are tested for Influenza viruses before treatment.

Diagnostic test methods for the influenza virus's detection and identification in respiratory specimens are mainly based on molecular assays, and antigen detection assays methods.

Molecular assay methods

Reverse transcription polymerase chain reaction (RT-PCR) is the method to detect influenza virus nucleic acids (RNA) in patient upper respiratory tract specimens. It is a rapid assay method with over 95% sensitivity and specificity. RT-PCR method target and identify different RNA genes for both influenza A and B viruses. This method is designed to detect virus RNAs of hemagglutinin (HA), Neuraminidase (NA), matrix (M), or nucleoprotein (NP) proteins [21]. Also, RT-PCR test method is capable to detect and discriminate between influenza A and B viruses' infection and identify specific seasonal influenza A virus's subtypes of (H1N1), (H3N2), and avian (H5N1) [22]. The methodology of this RT-PCR method (Figure 5) is based on transcribe virus RNA into cDNA by reserve transcriptase (RT) enzyme, followed by polymerase chain reaction (PCR) of virus cDNA amplification and detection. Reagents for this RT-PCR method are the enzyme reverse transcriptase, primers, DNA polymerase, and nucleotides. Replicated cDNA can be detected by specific detection methods (Figure 6) or instruments [23].

In summary, this test method has two steps, the first step virus RNA is reverse transcribed into complementary single strand DNA (cDNA), and the second step, specific segment of cDNA is amplified into double strand cDNA using, specific primers, nucleotide, and DNA polymerase enzyme [24]. Real time RT-PCR detection (Figure 7) is currently favored for the detection of influenza viruses because it is simple quantitative assay method, with high sensitivity and capable to detect virus infection soon after the infection or even before the onset of the disease [25].

Immune assay methods

Immunoassay is a detection method of virus antigen for both viable and nonviable influenza A and B viruses. This method can differentiate between influenza A and B viruses' types, but cannot differentiate between viruses' subtypes [26]. This method detects the virus in nasal washes, nasal swabs, or throat swabs samples by targeting the virus hemagglutinin (HA) using specific developed antibody against this target. In summary, immunoassay assay methods are antigen detection assays employs a combination of specific antibody (Ab) to the

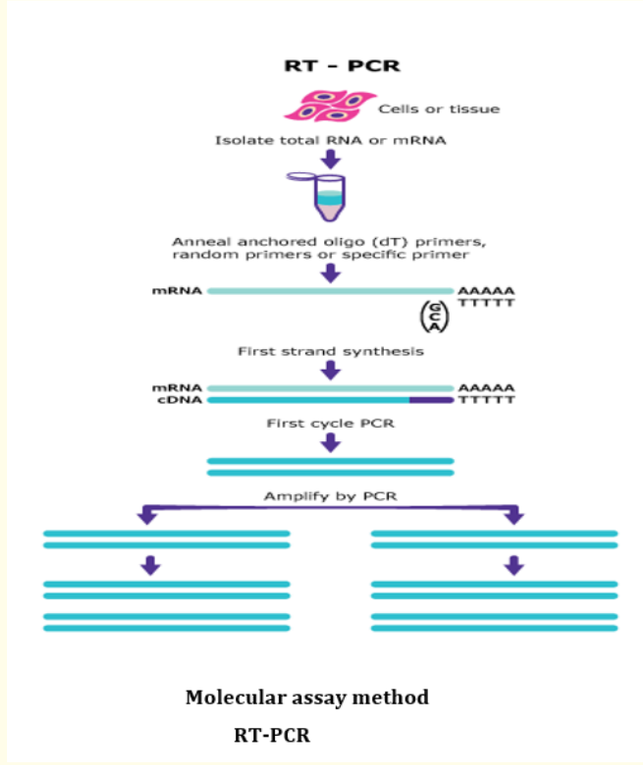

Figure 5: *The enzyme reverse transcriptase, virus RNA and the standard PCR reagents are used in this molecular assay method. The reaction mixture of enzyme reverse transcriptase and virus RNA is heated to 37^{0}C, which enables the production of virus cDNA from the virus RNA. In PCR step virus cDNA anneals to one of the primers leading to first-strand synthesis. Standard PCR proceeds and virus double strands DNA (dsDNA) is produced for virus cDNA detection on agarose gel electrophoresis.*

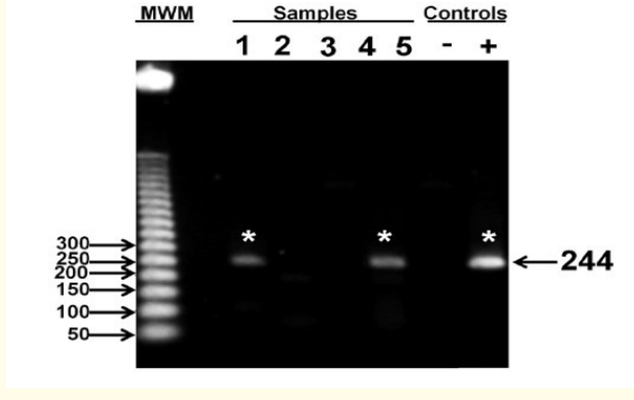

Figure 6: *Copy figure from Ramón Zazueta-Garcíaf., et al. [23] as an example for the detection replicated double stand cDNA on 2% agarose gel electrophoresis, after RT-PCR method for influenza A virus detection.*

Lanes*:*

0 is molecular weight as a marker (sizes from 50 bp to more than 300 bp).

1 and 4 are test samples with positive results for influenza A virus, showing the 244 bp size for influenza A virus replicated cDNA by RT-PCR.

2 and 3 are samples with negative result for influenza A virus.

6 is a negative control sample.

7 is Positive control sample show 244 bp band same as influenza A virus cDNA.

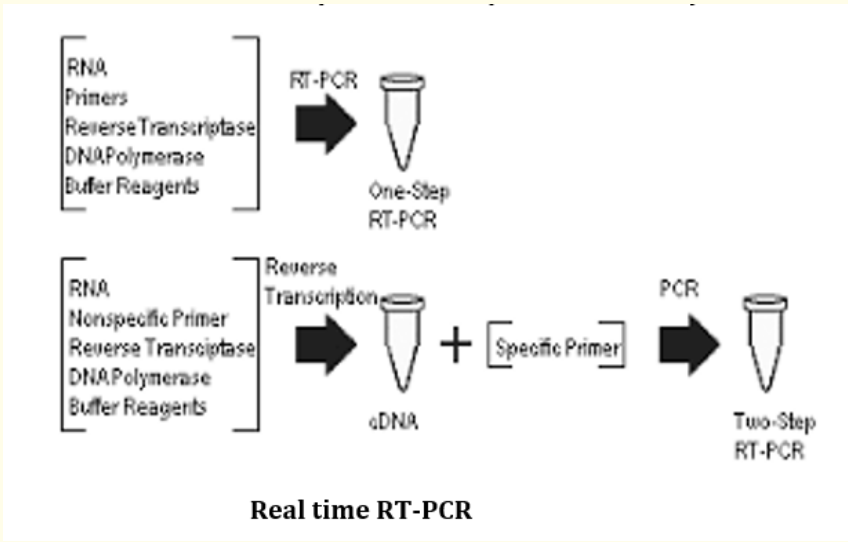

Figure 7: *Real-time RT-PCR. The top picture is a common virology test in one step diagnostic method that is widely used to quantify RNA transcript levels in virus. This direct method is a one-step method for both RT and PCR. The bottom picture is a indirect method, it is a two steps for RT and PCR.*

virus hemagglutinin (HA) glycoprotein and use affinity protein as a capture for the virus detection. These methods can detect influenza viral antigen in 10 - 15 minutes with moderate sensitivity and high specificity.

Enzyme Linked Immune Assay (ELISA) method [27] is antibody-antigen reaction and one of immune assay methods. ELISA use monoclonal antibodies (mAb) specific to influenza virus hemagglutinin (HA) glycoprotein as capture antibodies. Specific monoclonal antibodies (mAb) are affixed to wells in microtiter plate wells, test samples are added to plate wells and if virus antigen of hemagglutinin (HA) glycoprotein is present in the sample, will bind to the fixed capture monoclonal antibody (mAb) in plate wells, plate wells are washed to remove unbound particles, and second monoclonal antibodies (mAb) linked to enzyme are added to wells. This second monoclonal antibodies (mAb) will bind to other epitope in the captured virus antigen forming antibody-antigen-antibody sandwich (Figure 8). For virus detection enzyme substrate is added to wells and the enzyme-linked to second monoclonal antibody (mAb) will trigger a color change to identify the influenzas virus if presence in the sample (Figure 9).

Influenza viruses vaccines

influenza viruses is rapidly mutated (changes) specially for influenza A virus, and this require continue developing new versions of vaccines to protect people from seasonal influenza virus's infection [28]. Vaccination is the best method for the prevention and control influenza A and B viruses' infection and spreading in people. Vaccination can reduce influenza (flu) disease and lessen the severity of infection symptoms. World Health Organization (WHO) recommend yearly vaccination for people ages from six months to old ages, and for people with high risk of infection. Vaccines against influenza virus infection are generally safe but sometime cause temporary side effects such as muscle pains or feeling tiredness and It is estimated that 5 to 10% of children might develop temporary fever after vaccination [29].

Most Influenza (flu) vaccines are produced using fertilized chicken eggs for influenza virus multiplication [30]. In addition, there is a cell-based process for flu vaccine production by growing influenza virus on mammalian cell cultured in bioreactors [31]. Cell-based process for flu vaccine production, does not use fertilized chicken eggs and it is preferred for people suffering from eggs allergies. Disad-

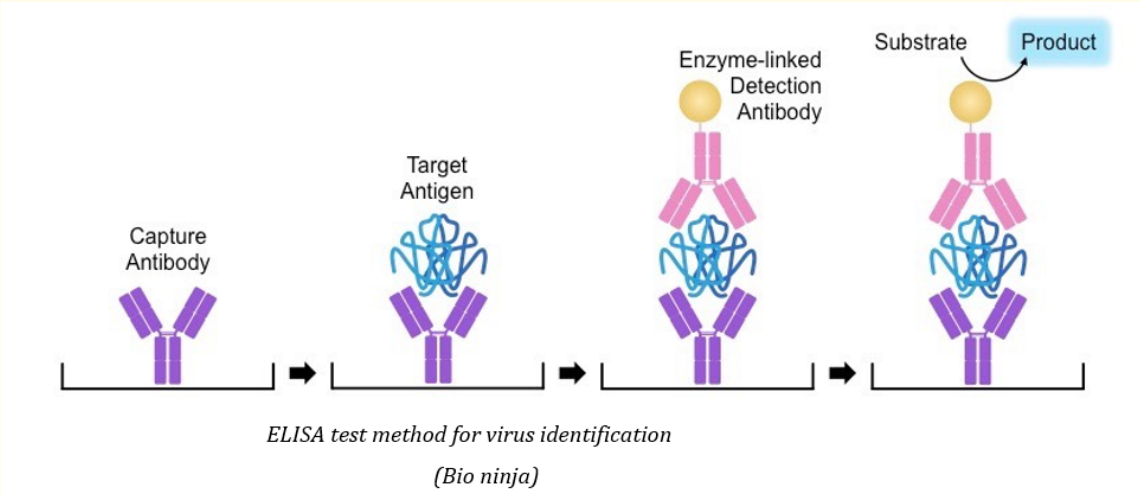

Figure 8: *Specific monoclonal antibodies (capture antibody) are affixed to wells in a microtiter plate, sample is added to the well and any antigen (virus) present will bind to fixed capture antibody, plates then washed to remove unbound particles, the second monoclonal antibody (detection antibody) linked to enzyme are added to the well, the detection antibody will bind to captured antigen (virus) creating a sandwich (antibody-antigen-antibody), the enzyme substrate is added to the well and the enzyme -linked to detection antibody will trigger a color change in the microtiter well to identify the presence of an antigen (virus).*

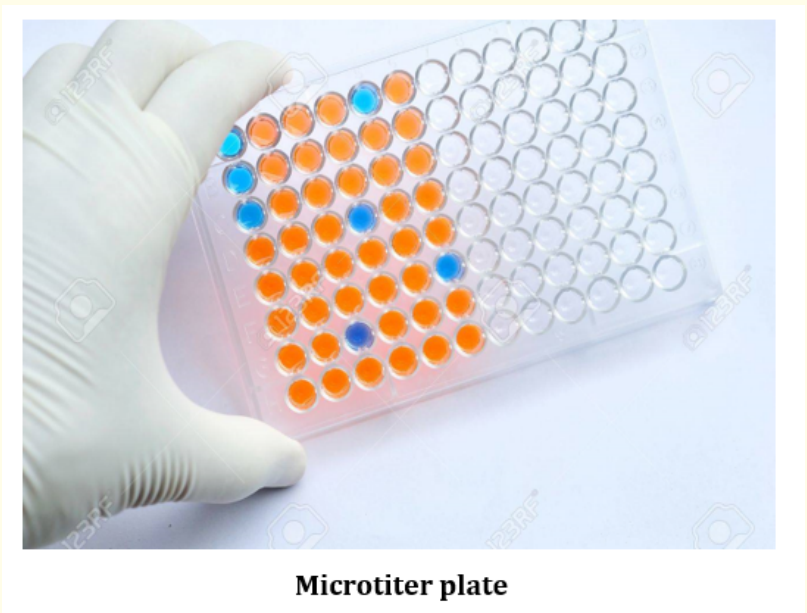

Figure 9: *Microtiter plates are used to perform the antibody/antigen test such as ELISA test method. Visible colored wells in this enzyme reaction with the substrate indicates the quantity of antigen in the sample. Darker color indicates higher antigen reactivity (virus concentration).*

vantage of cell-based technology is a tedious process in growing mammalian cells in bioreactors under aseptic conditions before infecting mammalian cells with influenza virus strain (s) for virus replications (multiplication). Other influenza (flu) vaccines production process is recombinant DNA technology [32]. Advantages of this recombinant DNA technology is eliminating vaccine manufacturing from egg-based process as in the case of from cell-based process. In addition, recombinant DNA technology are flexible in manufacturing influenza (flu) vaccines in a short time in responds to seasonal and pandemic newly emerged strains (mutated) of influenza viruses. Currently, eggs-based technology still the main process worldwide for influenza (flu) vaccines production.

There are two types of influenza (flu) vaccines that are widely available for people vaccination. These are inactivated influenza vaccines (IIV) and live attenuated influenza vaccines (LAIV). Traditionally, both IIV and LAIV are produced from fertilized chicken eggs-based process. These two types of vaccines are used to protect people against three different seasonal influenza viruses known by the name trivalent vaccine. This trivalent vaccine contains two common strains of influenza virus type A (H1N1) and type A (H3N2), plus one or two of influenza virus type B.

Inactivated influenza vaccine (IIV) is a non-viable influenza virus, killed by formalin or by β-propiolactone treatment after the virus multiplication in fertilized chicken eggs and harvested. IIV is demonstrated to be safe for both children and adults [33], administered annually by injection during seasonal influenza endemic.

Live attenuated influenza vaccines (LAIV) is attenuated or weakened viruses, approved for use only for people ages from 2 to 49 years old with no underlying medical conditions [34], administer annually as nasal spray during seasonal influenza endemic.

It is Important to highlight that both influenza vaccines IIV and LAIV contain adjuvants. Adjuvants are substances such as aluminum salts or emulsions (oil in water) that is added to vaccines to boost and lengthen body's immune response against influenza virus. adjuvants are normally added for any vaccine manufactured for use against any other microbial infection [35].

Immune response to influenza virus infection

Immune response to influenza virus infection is mediated by antibodies to the viral surface antigens such as hemagglutinin (HA) and neuraminidase (NA). This immune response is induced by virus infection or can be induced by vaccination, as a way to protect the host against reinfection with the same virus or against any antigenically similar viral strain. Secretary immunoglobulin A (sIgA) is the mucosal antibody response in the upper respiratory tract protect host from influenza virus infection [36]. Serum antibodies response are immunoglobulin M (IgM) and immunoglobulin G (IgG). These serum antibodies protect lower respiratory tract from influenza virus infection [37]. In the case infection these three antibodies responses of sIgA, IgM and IgG, are secreted by host plasma cells mainly against influenza virus surface glycoproteins of hemagglutinin (HA) and neuraminidase (NA) to neutralize influenza virus infection as a way of protection from virus infection mechanism. B lymphocytes (B-cells) are the most recognizable defense cells for their extending their lifespan as well as their ability to secrete large amounts of these three humoral antibodies of sIgA, IgM and IgG as a defense mechanism against influenza virus's infection. Serum antibody IgM levels peak in the host blood circulation after 2 weeks of infection and then start to decline, while the antibody IgG can be detected in the host blood circulation after 14 days of virus infection as a second response to virus infection [38]. Cytotoxic T-cell (CTL) is cellular immune response to influenza virus internal antigens proteins such as virus's matrix (M) and nucleoprotein (NP). This cellular immune response is to eliminate virus infection and, for the patient to recover from flu illness (Figure 10). Cytotoxic T-cells (CTL) are detectable in-patient blood circulation after 6 to 4 days of the virus infection and disappeared after 21 days of the virus infection or after vaccination [39]. Other cellular immune response is T-helper cells which plays an important role in stimulating humoral antibodies production of sIgA, IgM, and IgG against influenza virus (Figure 11). These T helper cells recognize influenza virus antigen (epitopes) of virus matrix (M) and Nucleoprotein (NP) to develop memory B-cells [40]. These memory B- cells circulated in host blood is a reminder if the same strain of influenza virus infected the host for the second time during the person life span. Upon a person reinfection with the same strain of influenza virus for a second time, it reactivates the patient immune systems of sIgA, IgM and IgG secretion against the same oldest virus infection as a defense mechanisms response [41].

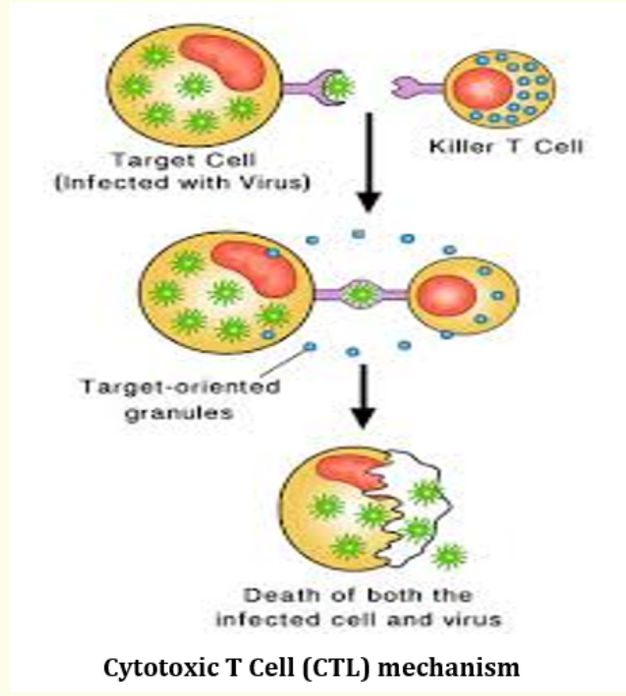

Figure 10: *Cytotoxic T cells (CTL) have special receptors (TCRs). TCRs are proteins on the surface of CTL. TCRs can specifically recognize a particular antigenic peptide from virus infection presented on the surface of Major Histocompatibility Complex (MHC) class I of target cells. TCR signal to CTL to releases cytotoxic factors to destruct (kill) the infected host cell and virus preventing the patient from virus invasion.*

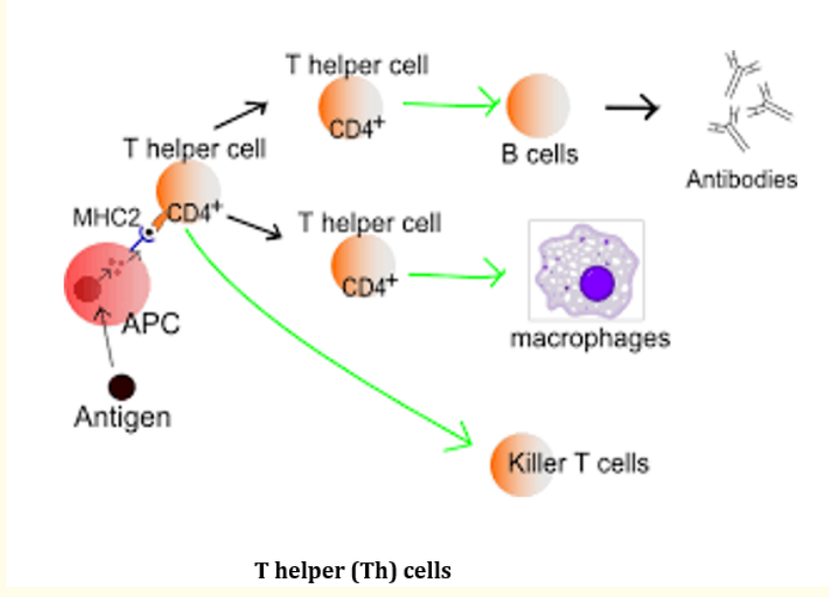

Figure 11: *T helper (Th) cells are CD4+ and are the most important cells in cell immunity responses, it helps activate B cells to secrete antibodies, help macrophages to destroy ingested virus or microbes, and activate cytotoxic T cells to destruct (kill) infected host cells.*

Serology test methods for antibody assay

Serological test methods are used for serum antibodies titer. These serology methods are hemagglutination inhibition assay (HAI), virus neutralization assay (VN), single radial hemolysis (SRH) method, and Enzyme linked Immunosorbent (ELISA). These methods are quantitative or qualitative assay for the detection of influenza virus's specific antibody responses:

1. **Hemagglutination inhibition assay (HAI):** Commonly used to detect the presence of influenza virus hemagglutination (HA)-specific antibodies as immune response in patient serum following influenza virus infection or vaccination. This assay is based on the ability of hemagglutinin (HA)-specific antibodies in patient serum to prevent the attachment of influenza virus to blood cells (erythrocytes). The source of these blood cells used in this test can be from human, birds, or animals. Patient serum at highest dilution that is capable to prevents complete hemagglutination of blood cells (erythrocytes) is called Hemagglutination inhibition assay (HAI) titer of the serum [42].
2. **Virus neutralization assay (VN):** Also, used to measure virus-specific antibodies as immune response following influenza virus infection or vaccination. This assay method is based on the ability of virus-specific antibodies in patient serum to neutralize influenza virus by preventing the infection of host cell culture (tissue culture). The highest serum dilution at which influenza virus infection to cell culture is completely blocked is considered the virus neutralization (VN) titer of the serum [43].
3. **Single-radial-hemolysis (SRH):** Also, used to measure virus-specific antibodies as immune response to influenza virus haemagglutinin, following influenza virus infection or vaccination. It is based on the passive hemolysis of influenza virus-treated blood cells (erythrocytes) by antibody in patient serum, and complement [44]. This method is considered to be valuable assay method in epidemiological studies for detecting newly emerged influenza virus variants.
4. **ELISA (enzyme-linked immunosorbent assay**): This method is a plate-based assay technique for detecting antibodies such influenza virus' antibody in patient serum. In this method virus antigen such as hemagglutinin (HA) is immobilized to a solid surface, patient serum for antibody testing is added, and a secondary antibody, conjugated to an enzyme or other detection molecule, is then added to bound to the first antibody (Figure 12). This method can be qualitative or quantitative assay method for the antibody assay in patient serum against influenza virus infection [45].

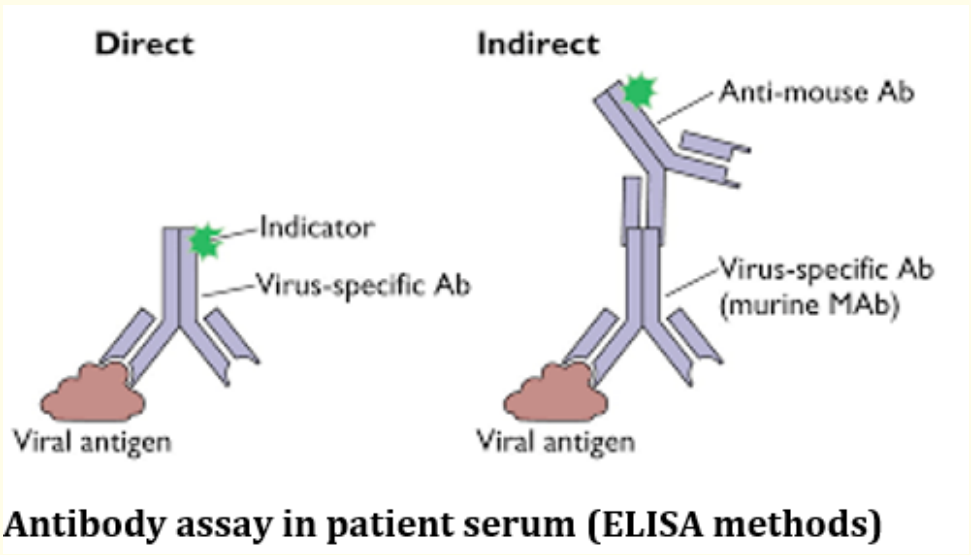

Figure 12: *Direct method: the virus antigen is immobilized to the surface of the multi-well plate and detected with an antibody specific conjugated with detection molecule. Indirect method: the virus antigen is immobilized to the surface of the multi-well plate. a primary antibody specific for the antigen binds to the target, and a labeled (conjugated with detection molecule) secondary antibody against specific to the primary antibody binds to the primary antibody for detection.*

Influenza virus (flu) infection treatments

Many healthy people with influenza (flu) symptoms do not require treatment from influenza virus infection, all these people need are drinking lot of fluid and resting in bed. In the case of patients having sever infection or with health complication due to preexisting health conditions or in old ages, family doctor may prescribe antiviral medications.

Antivirals drugs are developed based on viruses cannot reproduce (multiply) on their own but propagate by subjugating in a host cell to produce copies of themselves. Understanding the virus mechanism of infection assist researchers to develop effective antiviral drugs for patient's treatment [46]. Developing Influenza virus drugs are similar to the developing vaccines for influenza viruses. Both are based on targeting influenza viruses' glycoproteins of hemagglutinin (HA) and the neuraminidase (NA). These two influenza viruses' glycoproteins mediate the attachment of the virus to the patient respiratory epithelial cells for internalization into host cells. Influenza antiviral drugs, helps in shortening the duration of flu illness by day or two with efficacy ranges from 60 to 90% in influenza (flu) symptomatic prevention when these antiviral drugs are given within 48 hours of flu symptoms onset.

Developed antiviral drugs for Influenza virus's infection are:

1. **Amantadine:** Is antiviral drug used for the treatment of respiratory infections caused by influenza A viruses. The virus bound to host cell and transport into the endosome located in host cell cytoplasm. Acidic PH in the endosome open the M2 protein ion channel for hydrogen ion (H^+) to open virus envelop, and release virus genetic materials (RNAs) into host cell nucleus for virus RNA transcription and translation. Amantadine mechanism is inhibiting the virus M2 protein function in releasing virus genetic materials [47]. Amantadine is not effective on influenza B viruses due to the absence of M2 protein in influenza B virus structure. Amantadine hydrochloride is available in capsules for oral administration.
2. **Zanamivir (Relenza):** Is a viral neuraminidase inhibitor. Neuraminidase (NA) cleaves neuraminic acid component of sialic acid in the host respiratory epithelial cell. This host sialic acid is the receptor for the virus hemagglutinin (NA). Binding virus hemagglutinin to its host cell receptor (sialic acid), enhance the virus ability to infect host cells, and facilitate viral infection**.** The antiviral Zanamivir (Relenza) is neuraminidase inhibitor block influenzas virus ability to infect and spread into hos cells [48]. Zanamivir (Relenza) administered by oral inhalation and is antiviral effective for the infection by influenza A or B viruses.
3. **Oseltamivirare (Tamiflu):** Is also, neuraminidase inhibitor [49], has the same mechanism as Zanamivir (Relenza). Oseltamivirare (Tamiflu) is also antiviral for the infection by influenza A or B viruses. Oseltamivirare (Tamiflu) is available in capsules or oral suspension and is taken orally.
4. **Peramivir (Rapivab):** Is also, neuraminidase inhibitor [50], has the same mechanism as Zanamivir (Relenza), and Oseltamivirare (Tamiflu). Peramivir (Rapivab) is antiviral for the infection by influenza A or B viruses. It is administering via intravenous infusion for 15 to 30 minutes.
5. **Baloxavir (Xofluza):** Is a polymerase acidic endonuclease inhibitor. Influenza viruses have polymerase complex consist of three protein subunits. These subunits are polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA). These three polymerases complex is essential for influenza virus replication in host cell's nucleus [51]. The PB2 subunit binds to host cellular pre-messenger RNA (mRNA) and cleaved by the virus endonuclease PA subunit. These two steps allow by RNA polymerase of PB1 subunit to transcribe viral messenger RNA (mRNA). Baloxavir (Xofluza) inhibit endonuclease activity in PA subunit, blocking virus RNA replication in host cells [52]. Baloxavir (Xofluza) is taken orally, administered within 48 hours of the appearance of influenza virus (flu) symptoms.

Influenza (Flu) vs. Covid-19

COVID-19 is the name of the pandemic disease caused by the newly emerged virus SARS-CoV-2 strain. This2020 pandemic virus dramatically disrupted every day social and economic pattern of societies around the world [53]. Both flu and covid-19 are respiratory disease transmitted by person to person contact or by touching dry surface contaminated with respiratory droplets. Symptoms for both diseases are overlapped with 5% mortality rate from covid-19 illness comparing to 0.1% mortality rate from flu illness. The secondary bacterial pneumonias after flue illness is the major cause of death after the infection with influenza viruses. The similarity in symptoms

for both diseases Influenza (Flu) and Covid-19 making difficulties in distinguish between these two illnesses based on symptoms alone. In the flu season laboratory testing is a reliable approach and diagnostic tools are very important to distinguish between these two illnesses for the selection of proper treatment. Illness prevention from these two infections are the same, includes social distancing, wash hands, and wear masks when it is necessary. These personal hygiene's are essential to stop these two virus's infections and spreading into pandemic.

Discussion

Influenza (flu) is acute respiratory disease results from the infection with influenza viruses that infect and destruct cell lining of the upper respiratory tract, trachea and bronchi. Influenza virus's morphology are heterogeneous in size and shape, but generally are spherical or ovoid shape. Influenza viruses are enveloped (coated) viruses belonging to Orthomyxoviridae family. Virus outer layer is a lipid membrane which is taken from the host cell in which the virus infects and multiply. On the virus surface (lipid membrane) are glycoproteins of hemagglutinin (HA), and neuraminidase (NA). These two glycoproteins play important roles in the virus internalization into host cells, triggered the host immune response against the virus infection, used to determine the subtype of influenza viruses, and used as a target to develop vaccines and antiviral drugs for people protection and treatment respectively. Under the virus lipid membrane is the viral matrix (M1) protein which forms a shell that give the virus rigidity and shape. Within the interior of the virion are the virus genetic materials of antisense single-strand RNAs (ssRNAs) segments. Each RNA segment is joined with several proteins of PB1, PB2, and PA. Viral Nucleoprotein (NP) is another interior protein (capsid) which encapsulates the negative strand viral RNAs.

Many influenza virus strains isolated in 1930s and 1940s. All these isolates proved to be antigenic variants when compared to the first strain that was isolated from human. The first influenza virus isolated in 1933 was named Influenza A virus, the second isolated in 1940 was named influenza B virus and the third isolated in 1949 was named influenza C virus. These three Influenza A, B, and C viruses are currently determined by the nucleus of the virus. Since the discovery of influenza viruses' many new antigenic variants of influenza, A and B viruses have emerged and are the frequent recurrence of the epidemic influenza (flu) disease reflects the genetic variability of these two influenza types. Influenza C virus proved to be rarely causing clinical disease and has not been responsible for any pandemic or epidemics influenza (flu) diseases.

Influenza A viruses are divided into **subtypes**, based on the presence of the two virus surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). There are about 18 hemagglutinins (HA) variants, and about 100 types of neuraminidase (NA) variants. only H1, H2, and H3 variants for hemagglutinin (HA), and N1 and N2 variants for neuraminidase (NA), are the common and mainly found on the surface of human influenza viruses, Currently, there are two subtypes of influenza A viruses that are circulating among human populations, these are influenza A (H1N1) and influenza A (H3N2). Both subtypes are linked to influenza epidemics in human. Influenza A viruses in addition to infect human, also infects birds (avian), swine, horses, seals and dogs. The primary natural reservoir for all these subtypes of influenza A viruses is believed to be wild birds.

Pigs can be infected by influenza viruses from Avian, human, or swine, and can potentially be infected with influenza viruses from different species at the same time. If this happened, genes from these mixed viruses (**reassort)** can create a new different strain of influenza virus. Such newly different strains of influenza viruses circulate in pigs can infect people, if detected in human is called "variant" and denoted the letter "v" to the virus subtype. For example, the 2011 influenza A virus variant H3N2v was associated with exposure to pigs in agricultural fairs carried the virus matrix (M) gene from 2009 influenza A virus H1N1 pandemic. This Influenza A virus that named H3N2v is currently causing sporadic infection to human.

Influenza B virus is not classified into subtypes as influenza A viruses due to the lack of multiple hemagglutinin (HA) and neuraminidase (NA) variants that are found in influenza A viruses. The lack of these differences in hemagglutinin (HA) and neuraminidase (NA) structure limits the ability of influenza B virus to mutate. Influenza B virus cause the same symptoms of disease as influenza A viruses but does not cause pandemics, primarily infect humans and rarely infect animals.

Influenza C virus is also not classified by subtypes as the case of influenza B virus. Human infection by Influenza C virus causes mild upper respiratory tract illness, and very rare in causing lower respiratory tract complications. Influenza C virus causes epidemics and unlikely cause pandemic. Most human adults have been infected with influenza C virus during childhood.

Influenza A viruses are constantly mutating, and any small change to the genetic makeup of influenza strains is referred to *antigenic drift*, while a major change to the genetic makeup of influenza strains is referred to *antigenic shift.* These mutations occurred in the virus genes, and due to changes in the two virus surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). These two virus surface glycoproteins are the virus antigens that are recognized by the host immune system to promote immunological response against the virus infection. These antigenic drifts and antigenic shifts of influenza A virus's make it difficult to develop a universal flu vaccine to protect people from all influenza A virus's infection, and are the reason for continuously developing new influenza (flu) vaccines on yearly basis according to epidemiologist's prediction of influenza strains in the upcoming season.

Vaccination is the effective intervention to prevent the mortality and reduce morbidity from all microbial infection's viruses or bacteria. Influenza vaccines induce strain specific immunity and must updated annually based on predicted strains in the upcoming season. Currently influenza vaccines are manufactured by traditional method developed over 60 years ago using fertilized chicken eggs for influenza virus multiplication. These vaccines are developed based on the immunity to the hemagglutinin antigen that is highly mutated among different influenza A viruses that infect both human and animals. There are two types of influenza (flu) vaccines that are widely available in the market. These are inactivated influenza vaccine (IIV) and live attenuated influenza vaccine (LAIV).

Inactivated influenza (IIV) is A and B virus vaccines consist of purified viruses that has been chemically inactivated with formalin or β-propiolactone treatment. Inactivated influenza vaccine (IIV) is a trivalent vaccine contain inactivated two influenza A (H1N1), A (H3N2) and one or two of influenza B virus variants.

Live attenuated vaccine (LAIV) is also A and B virus vaccine of attenuated, A (H1N1), A (H3N2) virus and one or two of influenza B variants. It is manufactured based on strain of viruses replicates well at cold temperatures (25°C) and replicates poorly at host body temperature (37°C). This virus's temperature sensitivity is an advantage that the vaccine does not cause influenza (flu) illness and minimizes the risk to human infection. Live, attenuated influenza vaccine (LAIV) is a nasal spray vaccine that may be given to people ages from 2 to 49 years and is not recommended to pregnant women. The efficacy of LAIV demonstrated to be relatively higher than IIV vaccines, especially for children.

Influenzas (flu) vaccines, are seasonable needs for potential providing protection against newly yearly emerging influenza virus strains, including those that could cause influenza (flu) pandemic. Developing universal influenza vaccine (UIV) will eliminate yearly administer influenza (flu) vaccine and provide broad and durable protection from all influenza virus subtypes infections. Identification of broadly protective antibodies and cross-reactive T cells directed to influenza viral targets can be a promising prospect for the future developing such universal influenza vaccine (UIV).

The National Institute of Allergy and Infectious Diseases (NIAID) released the following universal influenza vaccine strategy [54]:

1. Understanding of influenza transmission, natural history, and viral pathogenesis of influenza infection.
2. Characterizing influenza immunity and immune correlates of protection through the study of immune responses to natural influenza infection and vaccination over time.
3. Support rational design of universal influenza vaccine (UIV) through the development and iterative clinical testing of immunogens, adjuvants, diverse platforms, and alternative vaccine delivery methods.

Currently, researchers identified multiple targets for developing universal influenza vaccine (UIV). One of these targets as a cross-protection vaccine is the stalk and the head of influenza virus hemagglutinin (HA). Virus hemagglutinin (HA) is a trimer protein that contains a globular head and a stalk domain. The globular head mediates binding to host cellular receptor sialic acids, while the stalk domain fuses the virus in the host cell membranes to allow the introduction of virus genes into the host cell nucleus. Hemagglutinin (HA) stalk domain is much more conserved compared to the globular head of hemagglutinin (HA). This can be an advantage in developing universal influenza vaccine (UIV) for triggering heterosubtypic Immunity to Influenza virus's infection, and meet the requirement of a broad

protection against all influenza virus's infection. Currently, a candidate for such universal influenza vaccine (UIV) based on the structure and function of the stalk and the head of influenza virus hemagglutinin (HA) glycoprotein have been developed and are under clinical trial investigation [55].

Conclusion

There is a hope for future developing a universal influenza vaccine (HIV) administer once in a single dose of immunization that can provide durable protection for all age groups against multiple influenza strains, including those that might cause a pandemic.

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