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Chronicles of Influenza A (H3N2): From Diagnosis to Epidemiology, Antigenic Drift, Vaccine Effectiveness & 50-Year Evolutionary Trends.

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Abstract
Influenza virus types A and B are responsible for major illnesses in humans, with type A being the only virus that causes pandemics due to its surface proteins H and N. H1N1 and H3N2 subtypes of influenza A have caused pandemics in the past, with H3N2 changing rapidly both genetically and antigenically. The review discusses various methods for detecting H3N2 virus, including conventional serological methods, molecular biology, and biosensors, all aimed at improving specificity and sensitivity. Real-time PCR is considered the gold standard test among molecular methods, while biosensors offer simple, rapid, highly sensitive, and specific detection of H3N2. The review also covers the classification and principles of various H3N2 biosensors

The Influenza A (H3N2) virus has been causing significant health issues for over 50 years. Its constant genetic and antigenic changes have made it a challenge to diagnose and control. This review explores the latest diagnostic techniques, including conventional serological methods, molecular biology, and biosensors, with a focus on improving specificity and sensitivity. The article also covers the virus's epidemiology, including its classification and evolution over the years. Additionally, the review discusses the challenges posed by antigenic drift and how it affects vaccine effectiveness, highlighting the importance of continuous updates to vaccine components. Whether you're a healthcare professional, researcher, or simply interested in learning about the latest developments in the field, Chronicles of Influenza A (H3N2) offers a comprehensive overview of this fascinating and complex virus.

Chronicles of Influenza A (H3N2) is a comprehensive review that discusses the diagnosis, epidemiology, antigenic drift, vaccine effectiveness, and 50-year evolutionary trends of the influenza A (H3N2) virus. The influenza virus is classified into four types, but type A and B are responsible for major illnesses in people, with influenza A being the only virus responsible for pandemics. This review focuses on the H3N2 subtype of the influenza A virus, which has been known to cause many flu pandemics.

The H3N2 subtype tends to change rapidly, both genetically and antigenically, and has evolved to form many separate, genetically different clades that continue to co-circulate. These changes have made it challenging to diagnose and vaccinate against the virus. This review describes traditional serological methods as well as advanced methods of molecular biology and biosensors for detecting H3N2 viruses. It discusses the advantages and disadvantages of each method and emphasizes the importance of improving specificity and sensitivity.

The review also discusses the evolution of the H3N2 virus over the past 50 years and its impact on public health. The virus has added numerous N-linked glycans to the surface of the HA protein, increased the overall net charge of the HA molecule, and altered its preferences in receptor-binding, among other changes. These mutations have made it difficult to predict which strains will dominate and require vaccine updates.

The review concludes by highlighting the need for collaboration between multiple disciplines of science to study the changing behavior of the virus. It emphasizes the importance of biosensors for fast and reliable detection of the virus, which can save lives and reduce the economic burden caused by pathogenic infections. Overall, Chronicles of Influenza A (H3N2) is a valuable resource for researchers, healthcare professionals, and policymakers seeking to understand the evolution and diagnosis of the H3N2 virus.

Keywords :

Influenza A (H3N2), diagnosis, epidemiology, antigenic drift, vaccine effectiveness, evolutionary trends, influenza virus, viral evolution, influenza surveillance, influenza vaccines, viral mutations, antigenic variation, influenza epidemiology.

INTRODUCTION :

Seasonal influenza is a significant burden on societies globally, with a large fraction of the population being infected annually and up to 650,000 deaths occurring each year (WHO, n.d.). The rapid evolution of influenza strains allows them to evade host immunity, enabling them to infect large portions of the population repeatedly. Due to this, limited public health resources need to be optimized efficiently to prevent infections[1].To effectively plan interventions for the spread of influenza viruses in urban areas, it is crucial to understand the dynamics of how the virus spreads between individuals. Although incidence and prevalence data can provide some insight into these dynamics, they do not provide information about the epidemiological relationship between individual cases. Therefore, it is essential to have a deeper understanding of the drivers behind the spread of seasonal influenza to develop effective interventions. The use of phylogenetics is a valuable tool in understanding the epidemiological connections between individual cases of influenza. This method involves reconstructing the evolutionary relationship between genetic sequences isolated from different infected individuals, sampled at different times. By analyzing the resulting phylogenetic tree, which displays the relatedness of the samples and the elapsed time between them, we can approximate the transmission chain of the sampled cases. This approach enables us to quantify the epidemiological dynamics that led to the observed phylogenetic tree,

using phylodynamic methods. In this way, phylogenetics provides valuable insights into the spread of influenza and can aid in developing effective interventions[2]. Respiratory illness caused by influenza affects a significant portion of the global population each year, with infection rates ranging from 5 to 15% (WHO, n.d.).

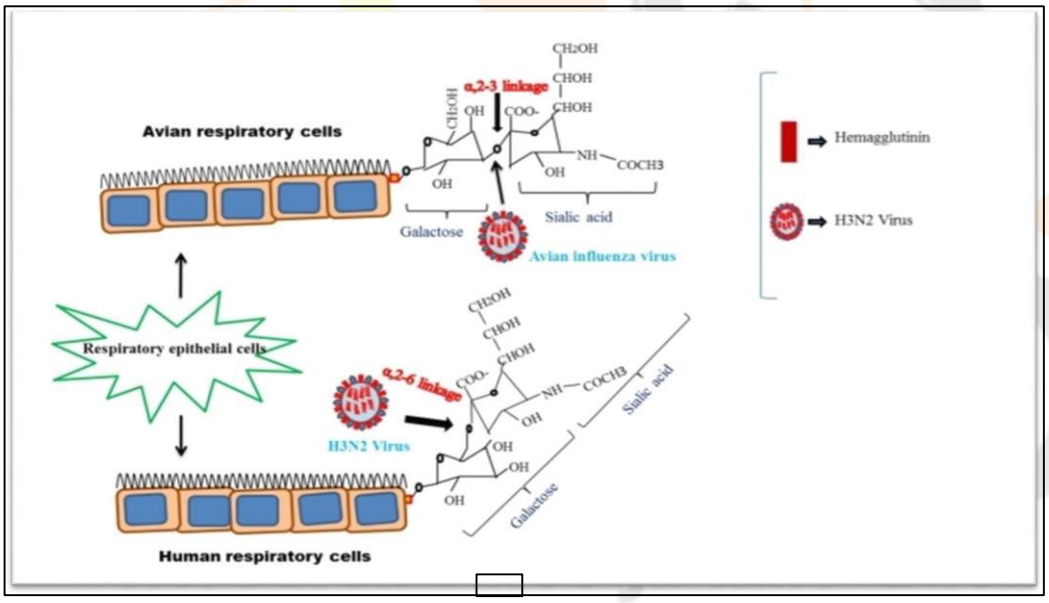


fig : Sessional Influenza.

This infectious disease is associated with high mortality rates worldwide.The latest report from the World Health Organization (WHO) reveals that cases of Influenza A(H3N2) viruses are widespread and affect over 30% of the global population (WHO update362). The WHO also estimates that these infections lead to 250,000–500,000 deaths annually.The Influenza A(H3N2) virus has caused numerous outbreaks and was responsible for the pandemic in 1968. During this period, a genetic reassortment occurred in the avian influenza virus, which resulted in millions of deaths worldwide when it was introduced to the human population. The influenza virus is categorized into four types, namely A, B, C, and D, with types A and B causing illness. Among these, influenza A, which belongs to the Orthomyxoviridae family, is responsible for pandemics. Influenza A is further classified into various subtypes based on the presence of hemagglutinin and neuraminidase surface antigens.

The year 1968 is often remembered in the United States for a number of significant events, including the Vietnam War, assassinations of prominent leaders, and achievements such as heart transplant surgeries and manned space flights. However, less frequently remembered is the H3N2 pandemic that occurred during that same year. Although the morbidity and mortality associated with this pandemic was smaller than the 1918 H1N1 pandemic, the ongoing impact of the influenza A(H3N2) virus on public health has been significant. Since its emergence in 1968, this subtype has caused more severe annual epidemics than those caused by influenza A(H1N1) and influenza B viruses. In this review, the authors reflect on the 1968 H3N2 pandemic, the continuing public health challenges from A(H3N2) virus, and the need for better prevention and control of seasonal and pandemic influenza.

Influenza viruses are known to evolve rapidly and require regular updates to vaccine components in order to provide optimal protection. The recent evolution of A(H3N2) viruses has proven to be particularly challenging for vaccine strain selection due to difficulties in antigenic characterization of circulating viruses and the propensity for candidate vaccine viruses to acquire antigenic changes during propagation in chicken eggs. To address these challenges, the World Health Organization convenes technical consultations to review data from global influenza surveillance systems and select vaccine reference strains. Delayed strain selection and changes in vaccine components can cause delays in subsequent manufacturing and distribution processes. In 2019, the selection of the A(H3N2) component was delayed to obtain additional data on changes in the distribution of A(H3N2) viruses, and a new clade 3C.3a candidate vaccine virus was ultimately recommended for the 2019-2020 northern hemisphere influenza vaccines.

JUSTIFICATION:

1. Influenza A (H3N2): This specifies the specific subtype of influenza virus being discussed, highlighting the importance of focusing on a specific strain for a more targeted analysis.

2. Diagnosis: This component emphasizes the process of identifying and confirming cases of Influenza A (H3N2) through various diagnostic methods, including laboratory testing, rapid diagnostic tests, and clinical evaluation.

3. Epidemiology: By including epidemiology, the title acknowledges the study of the distribution, patterns, and determinants of Influenza A (H3N2) in populations. It highlights the importance of understanding how the virus spreads, its impact on public health, and the implementation of control measures.

4. Antigenic Drift: This term refers to the gradual genetic changes in the viral surface proteins (hemagglutinin and neuraminidase) that occur over time. By including antigenic drift, the title recognizes the evolutionary process of the influenza virus and its implications for vaccine development and effectiveness.5

5. Vaccine Effectiveness: This component underscores the evaluation of how well influenza vaccines protect against Influenza A (H3N2) and their impact on reducing disease severity, hospitalizations, and mortality. It highlights the importance of monitoring and improving vaccine strategies.

6. 50-Year Evolutionary Trends: Including the evolutionary trends over 50 years emphasizes the long-term analysis of Influenza A (H3N2) and its dynamic nature. It acknowledges the continuous surveillance and research efforts to monitor the evolution of the virus, track emerging strains, and adapt vaccine formulations accordingly.

OBJECTIVES:

1. To review and analyze the current methods and advancements in the diagnosis of Influenza A (H3N2), including laboratory testing techniques and rapid diagnostic tests.

2. To examine the epidemiology of Influenza A (H3N2) by assessing its global prevalence, seasonal patterns, geographic distribution, and impact on different populations, including high-risk groups.

3. To explore the concept of antigenic drift in Influenza A (H3N2) by investigating the genetic changes in the viral surface proteins over time and their implications for viral evolution and vaccine effectiveness.

4. To assess the effectiveness of existing influenza vaccines, particularly against Influenza A (H3N2), by reviewing clinical trials, epidemiological studies, and real-world effectiveness data.

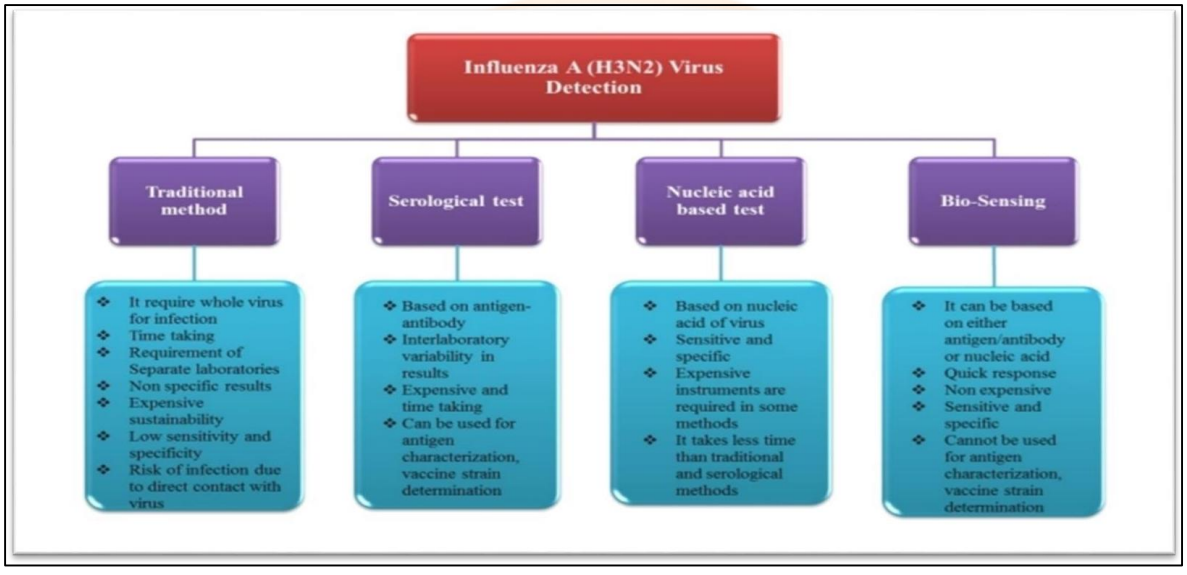
5. To analyze the 50-year evolutionary trends of Influenza A (H3N2) by examining historical data, genetic sequencing, and phylogenetic analysis to understand the patterns of viral evolution, emergence of new strains, and their impact on public health.

6. To provide insights and recommendations for improving surveillance strategies, vaccine development, and public health interventions related to Influenza A (H3N2) based on the findings of the study.

DETECTION TECHNIQUES OF INFUENZA A(H3N2)

Various techniques have been developed to detect influenza viruses, including traditional methods, serological methods, advanced quick methods, and bio-sensing methods. Traditional methods such as virus culture are still in use. Serological techniques include immunofluorescence assays, complement fixation, immunodiffusion tests, virus neutralization, hemagglutination, and rapid antigen testing. It has been found that nasopharyngeal swabs are more effective than nasal and throat swabs for rapid influenza detection. Advanced quick methods are based on molecular biology and include Real-Time PCR, multiplex PCR, Non-PCR-based RNA-specific detection, and conventional PCR. Bio-sensing methods involve optical biosensors, giant magneto-resistance biosensors, aptamer-based biosensors, and electrochemical

biosensors. In this review, we discuss all available methods for detecting H3N2, with a focus on bio-sensing methods. We previously reported on various detection methods for H1N1.



a) **FIG : Direction Of Influenza Virus**

METHODS

a) LABORATORY METHODS

During the enrollment process of a study, combined nasal and oropharyngeal swab specimens were collected from participants (with nasal swab specimens only collected from children under 2 years old). These specimens underwent real-time reverse-transcriptase polymerase chain reaction (RT-PCR) testing to identify the presence of influenza A and/or B viruses. Influenza-positive specimens with high levels of influenza virus RNA (defined as an RT-PCR cycle threshold value ≤30) were sent to the CDC for genetic characterization, where full-length hemagglutinin sequences were obtained through whole-genome sequencing. The resulting sequence data were then uploaded to the Global Initiative on Sharing All Influenza Data, and the viruses were classified into hemagglutinin clades based on phylogenetic analysis. Participants who tested positive for influenza were designated as case patients, while those who tested negative were considered noncase patients.

b) INFLUENZA VACCINATION HISTORY

The 2018-2019 northern hemisphere influenza vaccines were recommended to include virus strains such as A/Michigan/45/2015 (H1N1) pdm09-like, A/Singapore/INFIMH-16-0019/2016 (H3N2)-like, B/Colorado/06/2017-like virus (Victoria lineage), and B/Phuket/3073/2013-like (Yamagata lineage) viruses for quadrivalent vaccines. To determine vaccination status, participants, including children aged <9 years who require 2 vaccine doses during their first vaccination season, were considered vaccinated if they received at least one dose of any seasonal influenza vaccine at least 14 days prior to illness onset, according to medical records and registries at the Wisconsin site or medical records and self-report at the Michigan, Pennsylvania, Texas, and Washington sites.

c) STATISTICAL ANALYSIS

In this study, participants with inconclusive RT-PCR results, influenza-negative participants enrolled before periods of local influenza circulation, and participants vaccinated 14 or fewer days before self-reported illness onset were excluded from VE estimates. Descriptive statistics were calculated separately for influenza case patients and noncase patients, including medians of continuous variables and distributions of categorical variables. The test-negative design was used to estimate influenza VE, which compares the odds of testing positive for influenza among vaccinated versus unvaccinated persons. VE was considered as the relative difference in influenza risk between vaccinated and unvaccinated participants, expressed as a percentage and calculated as (1 – OR) × 100, where OR is the odds ratio for influenza among vaccinated compared with unvaccinated persons. The 95% Cis for VE were calculated as 1 – CIOR, where CIOR is the CI of the OR estimates. Estimates were adjusted for network site, sex, age, race/ethnicity, selfreported general health status, interval from illness onset to study enrollment, and biweekly interval. VE was estimated for any influenza-associated illness and separately for illness due to influenza A(H1N1)pdm09, A(H3N2), and A(H3N2) clade. The subtype/clade-specific estimates excluded patients infected with other influenza subtypes/clades.

d) SEROLOGICAL METHODS

Serological tests are commonly used to diagnose the immune response to influenza virus. These tests include various assays such as hemagglutination inhibition assay (HAI), virus neutralization assay (VN), single radial hemolysis (SRH), complement fixation assay, enzyme-linked immunoabsorbent assay (ELISA), and Western blotting. However, the complexity of sample collection, such as paired serum samples, makes routine use of these tests not recommended. The first swab should be collected at the onset of infection, and the second swab must be taken 2-4 weeks post-infection. Although this test is cheap and simple, its specificity is not satisfactory according to the Centers for Disease Control and Prevention (CDC) in 2020.

e) VIRUS NEUTRALIZATION (VN) ASSAY

The following text describes a technique used to detect antibodies against viruses, either after vaccination or natural infection. This method is commonly used for detecting antibody titers against avian or seasonal influenza virus strains. The technique is primarily based on the ability of virus-specific antibodies to neutralize the virus, and the resulting virus neutralization titer is the reciprocal value of the highest serum dilution at which infection is blocked.

This test is highly sensitive, but requires specialized laboratory facilities, such as BSL2+ and BSL3 laboratories, and is not typically used for routine diagnostics Enzyme-linked immunoassay (ELISA)

ELISA tests have been widely used for diagnostic purposes since the 1990s, as they possess high sensitivity and specificity. However, compared to nucleic acid-based tests, the major drawback of this test is its lower specificity. Conventional ELISA tests rely on the interaction between a specific antigen and antibody, with a color change indicating the presence of an immunocomplex-enzyme linkage for viral detection. To improve the sensitivity of ELISA tests, some scientists have explored the use of europium and gold nanoparticles.

In recent years, various studies have reported on the development of ultra-sensitive immunoassays for the detection of the influenza virus. One such assay is based on the use of gold nanoparticles (AuNPs) to enhance the signal, which can detect as low as 10 plaque-forming units of isolated H3N2 and has a sensitivity up to 500-fold higher than other available kits. This assay is useful in determining the potency of new antiviral drugs against the virus present in a patient's respiratory tract. Other methods have also been developed, such as quantitative virus culture, which detects the viral nucleoprotein (NP) in virus culture plates by ELISA and has provided good results with different isolates of influenza A viruses. However, ELISA takes 2-3 days to determine the virus type, and a single sample cannot be performed with ELISA, which is typically used for batch testing to reduce the cost of the test. Despite its limitations, ELISA is a very specific test and has been used in clinical trials during influenza seasons when non-hemagglutinating influenza A viruses circulated.

f) NUCLEIC ACID-BASED TESTS

Nucleic acid tests (NAT) are a reliable alternative to serological assays for diagnosing viral infections. These tests use virus-specific RNA or DNA sequences instead of antibodies or antigens, making them more sensitive and specific. Unlike antibody-based tests, which require time for antibodies to reach detectable levels, NATs can detect viruses in earlier clinical samples. Several NATs are available for detecting influenza virus in humans, including nucleic acid sequencing-based amplification (NASBA), reverse transcriptase PCR (RT-PCR), loop-mediated isothermal amplification-based assay (LAMP), and transcription-mediated amplification. These tests typically take 2-4 hours to complete and have higher specificity and sensitivity than serological tests.

g) RT-PCR

The detection of influenza virus is crucial for timely and accurate diagnosis and treatment. Among the various detection methods, reverse transcriptase polymerase chain reaction (RT-PCR) is considered the most powerful tool due to its high sensitivity and specificity. This method involves the use of nested primers to detect and subtype influenza virus. The process includes extraction of viral RNA, reverse transcription to complementary DNA, and product amplification with fluorescent detection. Multiplex RT-PCR techniques allow for the detection of numerous respiratory viruses in a single reaction, and can differentiate between influenza A and B and various subtypes of A. This technique offers a quick and simple way for clinical diagnostics and viral observation. In addition, a commercialized dual priming oligonucleotidebased multiplex PCR method has been developed for the detection of influenza virus A, B, and subtypes of A (H1N1, H3N2). RT-PCR is also recommended by WHO for the detection of SARS-CoV-2.

h) LAMP

LAMP is a nucleic acid amplification technique that has been developed for the detection of various viruses. The technique utilizes Bst polymerase, which has high strand displacement activity, to amplify viral cDNA. Two sets of primers are designed to target six distinct regions on the viral genome. The presence of the virus is detected either by observing a change in color after adding SYBR green or by detecting the magnesium pyrophosphate by-product released during the reaction. This technique has demonstrated 100% sensitivity for detecting seasonal influenza A viruses of the H3N2 and H1N1 subtypes from clinical samples using primers for the matrix gene. LAMP has also been used to detect the SARS coronavirus. Recently, a colorimetric RT-LAMP was developed to detect SARS-CoV-2 using primers against the nucleoprotein gene. This technique showed a sensitivity and specificity of 97.5% and 99.7%, respectively. A color change from red to yellow indicated the presence of the virus.

i) NASBA

NASBA is an isothermal amplification technique that does not require PCR and uses a combination of three enzymes: RNAse H, T7 RNA polymerase, and avian myeloblastosis virus reverse transcriptase in a single reaction. It has been extensively developed for the diagnosis of seasonal influenza type A and highly pathogenic avian H7N9 and H5N1 influenza A viruses. Recently, Wang et al. (2013) developed a customized NASBA method called Simple Method for Amplifying RNA Targets (SMART) for the detection of seasonal H1N1, pH1N1, and Influenza A(H3N2) viruses. This isothermal technique utilizes single-strand DNA (ssDNA) probes as reporter molecules to capture specific viral RNA (vRNA) sequences, which are subsequently separated on a microfluidic chip under zero-flow conditions. The SMART test demonstrated an analytical sensitivity of up to 105 vRNA copies/ml, with a test sensitivity of 98.3% and specificity of 95.7% for the detection of influenza A viruses. The Influenza A(H3N2) virus-specific probe showed 95% sensitivity and 100% specificity.

BIO-SENSING TECHNIQUES

In recent years, biosensing techniques have emerged as a promising tool for the identification and diagnosis of viruses, utilizing three

main components: a biological component, an amplification/processing element, and a signal-transducing component. Biosensors can be based on a variety of components, including DNA, RNA, aptamers, PNA, protein, antigen-antibody, and cells, and can detect targeted pathogens with high specificity and sensitivity. Aptamers, in particular, are synthetic oligonucleotides or proteins that can bind specifically to target non-covalently, and have high binding affinity and specificity. They can be selected through an in vitro genetic selection strategy, and have been successfully used in diagnostics, imaging, and therapeutic applications. Notably, aptamers have been used in the detection and treatment of influenza virus infections, with examples of DNA aptamers targeting the H3N2 influenza virus and exhibiting antiviral activity in vitro. Biosensors have also been developed for the detection of H3N2 and SARS- CoV-2 viruses using various methodologies.

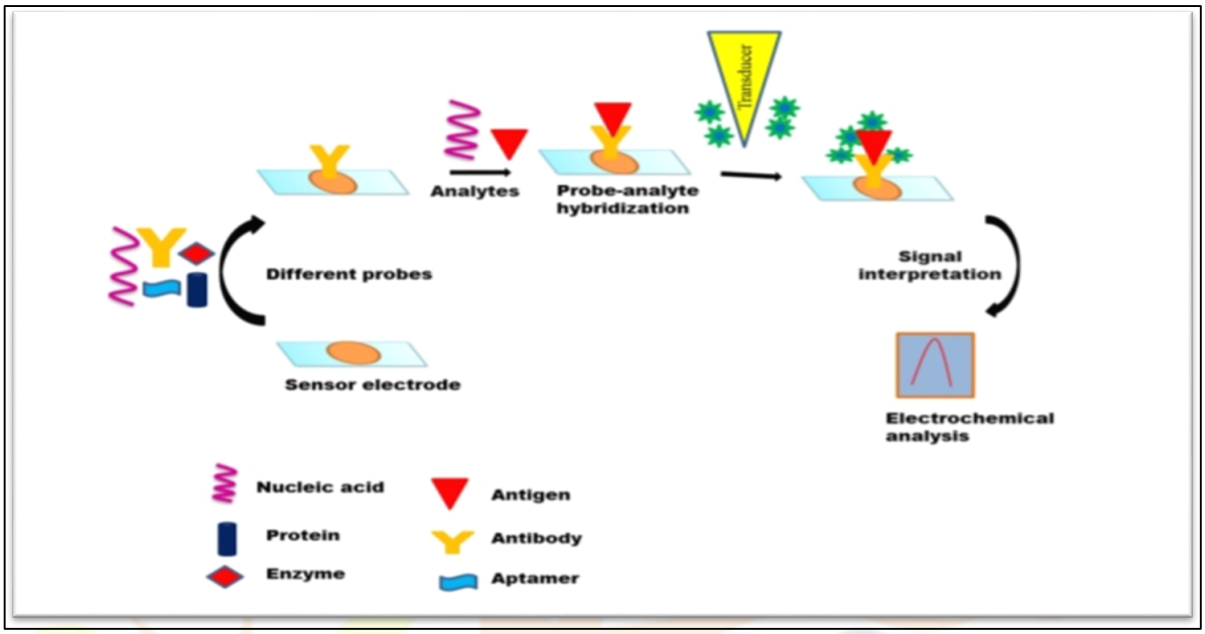


FIG. BIOSENSING TECHNIQUE

1. OPTICAL IMMUNOSENSOR

In order to detect the Infuenza A(H3N2) virus, a waveguide sensor was created using gold nanoparticles (AuNPs) as a tool for bio-nano sensor fabrication. AuNPs were chosen because they absorb visible light at 520 nm due to plasmons excitation, making them highly useful (Nagel et al. 2011; Tinguely et al. 2011). The sensor utilized an optical system with surface plasmon excitation, and a self-assembled monolayer with -COOH group combined with anti-HA antibodies was used against the H3N2 virus for better sensitivity and higher intense fluorescence. This prism-shaped fluidic channel sensor can detect 0.2 HAunitsml−1 of the influenza virus in just 1-2 µl of the sample (Nomura et al. 2013). Gopinath etal. (2013) also used AuNP-conjugated anti-A/Udorn/307/1972 antibody and a silicon-based sensing plate to identify both A/Udorn/307/1972 and A/Brisbane/10/2007 strains of the Infuenza A(H3N2) virus using a waveguide sensing method.

2. IMPEDIMETRIC BIOSENSOR

Influenza A(H3N2) virus detection has been the focus of many researchers, and different biosensors have been developed for this purpose. One approach is the use of glycan-based biosensors, which are composite carbohydrates involved in various physiological and pathological processes. Glycans have been shown to have specific selectivity for pathogenic subtypes, making them suitable viral receptors. An impedimetric biosensor was fabricated by immobilizing glycan on a self-assembled monolayer composed of OEG-COOH, reducing the cost and time for detecting the virus compared to using costly antibodies. This biosensor has a limit of detection (LOD) of 13 viral particles per 1 µL level. Another approach is the use of a boron-doped diamond sensor functionalized with polyclonal anti-M1 antibodies to recognize the common biomarker for the influenza virus, M1 protein. This sensor achieved an LOD of 1 fg/mL in saliva buffer, corresponding to 5-10 viruses for each sample in just 5 minutes.

3. QCM IMMUNOSENSOR

Researchers have employed the quartz crystal microbalance (QCM) to develop an immunosensor for detecting the influenza virus, as demonstrated by previous studies (Sasaki et al. 2007; Hewa et al. 2009; Owen et al. 2007). However, during the process of attaching the electrode surface, variations were observed in the detection of influenza antibodies. To enhance the sensitivity of the QCM immunosensor for influenza virus recognition, two different approaches were tested. In one approach, a crystal surface was used to detect the attachment of viral antigen and polyclonal IgG antibodies against the antigen for influenza A virus (VR-544, H3N2). In the other approach, gold nanoparticles (AuNPs) were utilized, owing to the QCM immunosensor's growing sensitivity for detecting influenza viruses. These methods were employed to detect three types of influenza viruses, namely H3N2, H5N1, and H1N1 (Hewa et al. 2009).

4. SERS IMMUNOSENSOR

In 2017, researchers developed a surface-enhanced Raman scattering (SERS) immunosensor that employed SERS spectroscopy to detect inactivated Influenza A(H3N2) virus (A/Shanghai/4084 T/2012). The immunosensor utilized a sandwich complex consisting of SERS tags, target influenza viruses, and highly SERS-active magnetic supporting substrates to enrich and separate viruses from complex matrices. The developed substrate enabled the detection of H3N2 down to 102 TCID50/mL with a good linear relationship from 102 to 5×103 TCID50/mL using a portable Raman spectrometer (Sun et al. 2017).

5. GMR-BASED BIOSENSOR

A biosensor based on Giant Magneto-Resistance (GMR) was developed using monoclonal antibodies of nucleoprotein (vNP) along with magnetic nanoparticles (MNPs). The presence of influenza virus allowed the binding of MNPs to the GMR sensor, which was directly proportional to the virus concentration. Unlike other magnetic particles, MNPs do not bleach and have no ferromagnetism property in biological samples. The GMR biosensor was able to detect swine influenza virus H3N2v at a limit of detection (LOD) of 1.5×102 TCID50/mL virus. Comparison of the GMR biosensor with ELISA showed that the GMR biosensor was more sensitive than ELISA.

6. APTAMER-BASED BIOSENSOR

A novel surface-enhanced Raman scattering-based aptamer sensor was developed for the detection of H3N2 virus. The sensor consisted of primary and secondary aptamers specifically created for the hemagglutinin of the H3N2 virus, attached to metal nanoparticles on a SiO2-covered silicon plate with silver zones. The sensor showed high sensitivity and specificity for detecting influenza strains, with a limit of detection of 10^4 virus particles per sample.

7. PLASMONIC CONTRAST IMAGING BIOSENSOR

A biosensor based on plasmonic contrast imaging was developed, which works by measuring the intensity difference between p- and s-polarization images at plasmonic excitation. This method improves the sensor resolution by eliminating system noise. The sensor was able to detect refractive index with a resolution of 4.36×10–7 RIU.

The plasmonic contrast imaging biosensor was also used for the detection of H3N2 influenza antibodies and DNA–DNA molecular binding with a detection limit of 8.6 nM (320 ng mL−1) and a sensor resolution of 38 nM, respectively.

LOCALIZED SURFACE PLASMON RESONANCE (LSPR)-BASED FLUORESCENT NANO SENSOR

A biosensor was developed for the detection of Infuenza A(H3N2) virus, which uses quantum dots (CdSeTeS) conjugated with anti-hemagglutinin antibody (anti-HA Ab) along with gold nanoparticles thiolated with l-cysteine and conjugated with anti-neuraminidase antibodies. Both antigens were targeted using separate binding agents, i.e., AuNPs and quantum dots. Upon interaction of antigen with antibody, localized surface plasmon resonance was generated from gold nanoparticles, which enhanced the immune-fluorescence signal of alloyed CdSeTeS quantum dots. The use of quantum dots along with AuNPs increased the fluorescent signal and sensitivity of the sensor for the sample concentration. The limit of detection of the sensor was found to be 10 PFU/mL for clinically isolated Infuenza A(H3N2) virus.

CONCLUSION AND FUTURE PERSPECTIVE

Since its emergence in 1968, the Infuenza A(H3N2) virus has undergone significant antigenic changes, such as the addition of multiple N-linked glycans to the HA protein, which has resulted in an increase in the overall net charge of the HA molecule, alterations in receptor-binding preferences, and changes in the ability of neuraminidase (NA) to agglutinate red blood cells during host entry. Moreover, genetic mutations have been reported, including N158-linked glycosylation in HA, HA F193S substitution, and HA T135K and/or I192T, which rapidly change the genotype/phenotype of the virus. (Allen and Ross 2018).

S.N	Detection method	Target	Time	L.O.D	Specificity	Sensitivity	References
1	Minor groove binder TaqMan assay	HA gene	–	16.5 standard DNA copies	–	–	(Wang et al. 2011)
2	Waveguide-mode sensor	HA antigen	–	86,103 PFU/ml	–	–	(Gopinath et al. 2013)
3	Optical biosensor	Virus	–	0.2 HAU/ml	–	–	(Nomura et al. 2013)
4	Europium nanoparti-cle-based immuno-assay	Nucleoprotein	–	$1.00\times10^{2.0}$ EID_{50}/ml	100%	90.7%	(Zhang et al. 2014)
5	RT-PCR	Viral RNA	20–30 min	–	>95%	100%	(Cui et al. 2016)
6	GMR biosensor	Nucleoprotein (NP)	–	1.5×10^{2} TCID50/mL	–	–	(Krishna et al. 2016)
7	Glycan based biosensor	Lectin	–	13 viral particles/µl	–	–	(Hushegyi et al. 2016)
8	Multiplex one-step real-time RT-PCR assay	HAgene	–	4.8×10^{1} copies per reaction	>95%	100%	(Cui et al. 2016)
9	LSPR nanosensor	HA, NA antibody	–	10 PFU/mL	–	–	(Takemura et al. 2017)
10	SERS immunosensor	Whole virus	–	$10^{2}TCID_{50}$/mL	–	–	(Sun et al. 2017)
11	Multiplex RT-PCR assay	NP protein	–	1×10^{0} $TCID_{50}$/100 µl	–	–	(Wang et al. 2017)
12	Antibody modified boron-doped dia-mond	Anti-M1 antibodies	–	1 fg/ml	–	–	(Nidzworski et al. 2017)
13	DFA	Viral antigen	2–4 h	–	80–100%	70–100%	(Dziąbowska et al. 2018)
14	LAMP	Viral RNA	2 h	–	100%	97.8%	(Dziąbowska et al. 2018)
15	SERS aptasensor	HA protein of whole virus	12 min	10^{4} virus particles per sample	–	–	(Kukushkin et al. 2019)

All methods detect a different analyte of the same virus at a different detection time with a limit of detection value

DFA direct fluorescent antibody, *GMR* giant magneto-resistance, *LAMP* loop-mediated isothermal amplification-based assay, *LSPR* localized surface plasmon resonance, *SERS* surface-enhanced Raman scattering

Table 1: A comparison of all analytical methods for detection of the Infuenza A(H3N2) virus is explained in the table

Different simulations are utilized for early prediction of virus mutations and antigenic drift in 3C.3a clades, which are currently circulating, leading to reported vaccine ineffectiveness and making people vulnerable to the virus. A study was conducted to assess the vaccine effectiveness among patients infected with Infuenza A(H3N2) virus during the 2018-2019 influenza season in the USA, using the Reverse Transcriptase polymerase chain reaction (PCR) for testing samples and sequencing the hemagglutinin gene segment for genetic analysis. The study showed a decrease in vaccine effectiveness against the current patients due to antigenic drift in the Infuenza A(H3N2) virus of 3C.3a clade currently circulating, and recommends an update in vaccine components. Phylodynamic simulations were used in another study to predict future virus strains that will dominate on earth, helping in making vaccine production fast without wasting time in strain selection. Thus, it is essential to modify diagnostic methods according to the new assorted or mutated virus for detection. Biosensors are providing fast results, good sensitivity, specificity, and are easy to perform compared to older time-consuming and laborious methods, some of which are infectious. With changing virus detection methods, biosensors are also evolving to catch the virus early and save human life and the global economic burden due to pathogenic infections. It is important to make a joint effort by collaborating with multiple disciplines of science to study the changed behavior of the virus as they evolve and change their receptor-binding ability, specificities, charge at proteins, virulence, and resistance against human immunity.

Abbreviation:

Influenza A (H3N2) Chronicles: Diagnosis, Epidemiology, Antigenic Drift, Vaccine Efficacy, and 50-Year Evolution

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Abbreviation:

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