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Review Article

Pandemic H1N1 2009 and Its Characterization: Tools and Techniques in Current Scenario

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Abstract

Background: Influenza A (H1N1) is the most pandemic diseases which affected the world's population. The aim of this review was to study about molecular diagnostic tools and techniques for early detection of pandemic Influenza A (H1N1) and other types of influenza viruses as well. The aim of this review was to study about molecular diagnostic tools and techniques for early detection of pandemic Influenza A (H1N1) and other types of influenza viruses as well. The current usage of the novel tools and technologies and various platforms are being discussed.

Conclusion: Different kind of technologies are developed to detect the Influenza viruses including Influenza A (H1N1) i. e. Real Time–Polymerase Chain Reaction(RT-PCR) and Gold standard Technology from different commercial assay which gives results within 4 to 8 hours for which the treatment of patient can start firstly. The other technology i.e. Culture technology, Rapid influenza Diagnostic test take minimum 2 to 3 days to give confirmation for infection where the chances of contamination is higher. In the case of RT-PCR, there is no chance of contamination of sample as well it save the time to delay to start the isolation and treatment of patient as fast as possible. Keywords: Pandemic H1N1 2009 Strain; Real Time–Polymerase Chain Reaction; Rapid Influenza Diagnostic Test; Epidemics; Gold standard Technology

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Introduction

The major cause of health concern is the large number of lives worldwide in every year are the infectious diseases (Ministry of Health Report 1920). These infectious diseases can either be new or rare infections which may occasionally or may be common infections which increasing the issues in our society. Influenza is the most common type of infectious diseases which cause by the different types of influenza that is Influenza A,B,C and also D. Human Influenza viruses have the three hemagglutinin subtypes, H1, H2 and H3 which have emerged as important pathogens. Recently, the influenza virus with subtype H5, mutated from H7, has also emerged as human pathogen and they are more lethal than the earlier strains [1]. They have a range of hosts that include pigs, humans and also birds.

In April 2009, a new strain of influenza virus A/H1N1, commonly referred to as "swine flu", began to spread in several countries around the world. The World Health Organization to quickly raises it s pandemic alert level to phase 5(29 April 2009) [2]. On 11th June, the alert was raised to phase 6 [2], which was indicating that full global pandemic was underway. However pandemic influenza A virus strains are often unpredictable because illustrated by the recent emergence of swine origin influenza A H1N1 virus which cause significant morbidity and mortality in immunocompetent adults.

Tools and technologies for the characterization of influenza viruses

Antigenic drift resulting from accumulation of point mutation in the viral genome generates novel variants that escape immunity to previous Influenza Strains causing annual seasonal epidemics. Genetic assortment between humans, swineoravianInfluenza strain has been shown to results in emergency of Influenza viruses with novel HA(Hemagglutinin) and NA (Neuraminidase) genes, against which the majority of human population lacks immunity. Diagnostic techniques and approaches that can rapidly and accurately detects newly emerging viral variants are required or quick initiation of antiviral therapy and prophylaxis to effectively control infection during seasonal and pandemic outbreaks. NATs (Nucleic acid amplification technique) have demonstrated high specificity and sensitivity for detection of Influenza viruses. However, they are less practical in resource-limited regions due to their high cost instrumentation complexity, requirement for well-maintained environment and highly trained professionals. A large number of low cost, portable, point of care RIDTs (Rapid Influenza Diagnostic Test) based on multiple mechanism been developed meet the demands for rapidly diagnosing epidemic Influenza in remote settings. Unfortunately, RIDTs (Rapid Influenza Diagnostic Test) have demonstrated variable sensitivity for the diagnosis of both seasonal and pandemic Influenza virus infections. Furthermore, most of the current FDA (Food and Drug Administration) licensed tests for Influenza A and B viruses but have a limited capability to further subtype Influenza A viruses. Hence newer approaches that are cost effective less labor intestine easy to perform, and have capacity to detect and differentiate Influenza viruses subtyping Influenza A viruses are currently a global public health requirement for the proper management of Influenza outbreaks.

Rapid influenza diagnostic test (RIDTs):

RIDT is also known as POCT (Point of care-testing). RIDTs (Rapid Influenza Diagnostic Test) are antigen based tests developed for rapid diagnosis of influenza virus infection in POC betting. These test use monoclonal antibodies that target the viral nucleoprotein employ either enzyme immunoassay or immune chromatographic (lateral flow) techniques. Available in dipstick, cassette, or card formats, RIDT scan be completed in less than 30 minutes with the results observed visually based on a color change or other optical signals. Dipsticks are placed directly in wells or tubes containing the respiratory specimen and the test kit extraction agent. Alternatively the nitrocellulose strip can be placed inside a plastic housing (cassette) or bound to thick paper (card) Several FDA approved RIDTs are for currently available on the market. Most of the test can either detect or distinguish Influenza A and B viruses (but cannot discriminate Influenza A and B), However none of the RIDTs can distinguish between the different Influenza A subtype. RIDTs have generally demonstrated high specificities (95-99%) for the detection of seasonal influenza virus infection. For diagnosis of seasonal Influenza infections, RIDTs have demonstrated variable assay performance with sensitivities ranging between 10%-70%, with up to 90% specificity compared to standard RT-PCR based analysis [3].

Effectiveness of rapid Influenza test

Commercially available RIDTS are reactive with the nucleoprotein of pandemic (H1N1) 2009 virus. However data regarding their sensitivity data are very limited based on preliminary results using clinical specimens, compared with RT-PCR. It is important to consider in each case that the several factors can effect sensitivity.

Detection of Influenza Outbreak using of RIDTS for public health purpose

In any setting, especially in institutions (hospitals, chronic care facilities, nursing homes), summer campus, school, cruise ships etc. RIDTs can be useful to identify influenza virus infection as respiratory outbreaks cause. Positive test for RIDT results from one or more ill person with suspected influenza can support decision to make infections presentation and measures to control the influenza outbreak.[4] Because of limited sensitivity of this test influenza virus infection is not excluded from the negative RIDT result as a cause of respiratory outbreak. The suspected person with influenza testing of respiratory specimens leads to increase the likelihood influenza virus infection detection if it is cause of the outbreak. The molecular assays techniques such as RT-PCR are used if outbreak cause is not detect and suspection of influenza is there (20) [4].

Sensitivity of the RIDT

It is proportional to the RIDT positive results of all positive "gold standard test" RT-PCR or viral culture. Fixed characteristic of a test; generally low to moderate (50-70%) for RIDTs. The sensitivity which is low for RIDT will produce negative results in some patients with influenza (false negative).

Specificity of RIDT

Specificity is proportional to the RIDT results which are negative of al negative "gold standard test" results (RT-PCR or viral culture). Fixed characteristic of a test; generally RIDT is very high (90-95%). The positive result is produce if RIDT has low specificity in those patients who don't have influenza (false positive).

Presentational strategies for hospitalized patients

The recommendation of testing of Influenza should be done for hospitalized patients with suspected influenza. If result of RIDT comes negative then also the treatment with antiviral should not be stopped because of the limited sensitivity of RIDTs. Then if the RIDT results are negative, just upon admission influenza patients, prevention for infection and measures of control should be implemented. Clinicians should understand that the influenza virus infection is not excluded if the influenza test is excluded, especially in the time from illness onset to collection is not more than 3 days. Or if, the patients has lower tract and his/her upper respiratory tract specimens tested. The clinical specimens from different respiratory site that is upper and lower respiratory tract can be collected for testing if influenza is suspected in the patient and can be collected on more than one day to increase the likelihood of influenza virus detection. The suspected of influenza is the intubated patients are there, but not yet confirmed then they should have endotracheal aspirate specimens. These should be done as a part of a broader surveillance strategy for influenza as discussion in prevention strategies for seasonal influenza in health care settings [4].

Use of RIDTs in clinical decision making

To diagnose and for treatment decision for patients in clinical settings such as whether to advice medications for antiviral, RIDTs can be used. Negative results of RIDTs do not exclude influenza virus infection in patients with sign and symptoms suggestive of influenza, however due to limited sensitivities of RIDT. Even if the test by RIDT comes negative, the treatment of antiviral should not be withheld from patients who are suspected with influenza if clinically indicated and indications by molecular assays of respiratory specimens for further influenza testing may be done. To implement the decision of antiviral treatment decision, testing is not needed for all the patients with same signs and symptoms suggestive of influenza. The outpatients with signs and symptoms consistent with the suspected influenza, especially during periods of activity of influenza in the community at the peak level, a clinical diagnosis can be made, once influenza activity has been documented in the geographic area or community [4].

Advantages of RIDTs

Rapid molecular assay and some commercially available molecular assays can produces results in reasonable time period to inform clinical management ranging from approximately 15-30 minutes to less than 1.5 hour. They are more sensitive and specific for detecting Influenza tests. They show simplicity in their use [5].

Disadvantages of RIDTs

Sensitivity of RIDTs to detect influenza B viral antigen is lower than for detection of influenza A viral antigen. Although specificity is high, false positive results can also occur especially when influenza activity is low. RIDT sensitivities are low to moderate approximately 50-70% which tells us that the false negative results are common. The false negative results can also occur when prevalence of influenza is high in the community that is typically when influenza season is on peak. Some of the RIDTs differentiate between Influenza A and B viruses while other does not. RIDT which gives us result on the type of Influenza virus, do not gives us any information on Influenza A virus subtype [5].

Cytokines in influenza virus infection

When the case of H1N1 infection becomes severe, the activation in excessive amount of the instinct immune system takes place. This results in the cytokine response in excessive, which is also known as cytokine storm. The cytokine response is the key benefactor to morbidity (a diseased state) and mortality (death). When the pandemic H1N1 influenza virus infection becomes much severe, it leads to the hyper activation of the pro- inflammatory cytokines. These pro-inflammatory cytokines are of following types: IL-6, IL-8, MCP-1, MIP-1β, GM-CSF and TNRF-1. The IL-6 plays very important role in this. If the H1N1 infection is in its milder form, then these (types of cytokines) are not visible. The individuals or patients who died because of confirmed pandemic influenza virus H1N1 showed the bronchiolar epithelial necrosis and peeling of skin(desquamation) as well as pattern of exudative diffuse alveolar damage. The results are congruous with the finding of the histopathology in necropsy of the patients with pandemic H1N1 infection [6].

Gold standard technique for H1N1 virus

Gold Standard Technique is the best technique to detect the Influenza Virus diagnosis for which the virus isolation in chicken embryos or tissue culture technologies but the procedure are time consuming which take 2-14 days before results are available [7]. The time saving technology which is known as Gold Standard Technique is using Reverse Transcriptase (RT) Polymerase Chain Reaction (PCR) and also helpful to detect the viral load in short time through isolation of RNA from samples [8-12]. RT-PCR is very sensitive and specific molecular method to detect Influenza virus from clinical samples [11,12].

One step RT-PCR

Duplicates of each RNA samples and standard were amplified by the QRT-PCR. The one RT-PCR (Ambion, Austin TX, UsA) with ROX addes as passive reference dye. The no. template controls and 2009 H1N1 negative specimens did not produce detectable fluorescence signal. The detection limit of the assay by QRT-PCR was assumed as 1x 10^1 RNA copies/ul whereas, in general, gel based RT-PCR is limited generally to detect to 1 x 10^2 copies/ul [13].

Use of qRT-PCR for clinical analysis

The type A Influenza Virus Real Time RT-PCR matrix screening assay is able to detect all viral isolates tested in the study, the H1N1 2009 QRT-PCR selectively detects only the novel pandemic H1N1 2009 Viral RNA [14].

Advantages of molecular assays

Rapid molecular assay and some commercially available molecular assays can produce results in a reasonable time period of inform clinical management (ranging from approximately 15-30 min. to less than 1.5 hrs.). Molecular assays are more sensitive and specific for detecting Influenza Viruses than other influenza tests (example: rapid influenza diagnostics tests, immune fluorescence, and viral culture). The likelihood of a false positive or false negative result is low and therefore, the interpretation of the result is less impacted by the level of Influenza activity in the community. Some, but not all molecular assays can distinguish between specific Influenza A Virus subtypes.

Disadvantages of molecular assays

Results of some RT-PCR and other molecular assays may not be available in a clinically relevant time frame to inform clinically management decisions. RT-PCR and other molecular assays may not be available in all outpatient or emergency room settings. For hospitalized patients, these assays are not always available on-site. Respiratory specimens may need to be sent to a state public health laboratory or commercial laboratory for RT-PCR. Therefore, although the test can yield results may be substantially longer. Most FDA-cleared molecular assays are not approved to test lower respiratory tract specimens RT-PCR and other molecular assays are generally more expensive than others Influenza tests. Some molecular assays may not specifically identify all currently circulating Influenza A Virus subtypes. Depending on the test, a negative result for one Influenza A Virus subtypes may not preclude infection with another Influenza A Virus subtypes. Some Influenza molecular assays being used are not FDA-cleared and an evaluation has not been performed to assess the accuracy of all available RT-PCR and molecular assays.

Why RT-PCR over end-point PCR for H1N1?

The aim of the present study was to compare RT-PCR and End-Point PCR with respect to their suitability for the analysis of gene expression in sample in which the number of cell is limited. For example;- in studies of H1N1 development and to determine the variability of the Real Time Reverse Transcription PCR assay. The sensitivity, specificity and rapid detection feature of RT-PCR were compared using cDNA standard. The Real time PCR was 100 folds more sensitive than End-Point PCR. The coefficients of variations (CV) for reverse transcription combined with real time analysis and consisting of mRNA isolation. End-Point PCR is more time consuming as it uses Agarose Gel Electrophoresis to detect the amplified PCR products, whereas, Real Time PCR I less time consuming as it can detect the amplifications during the run after each cycle. End-Point PCR has very poor resolution (min. 10 fold change) while Real Time PCR can detect very little change min.2 fold) due to the high resolution. End-Point PCR collects data at the end of the reaction while, RT-PCR collects data during the exponential phase. In End-Point PCR, detection of samples is done through Gel Electrophoresis and Amplification technology i.e. probe is not used in this PCR so fluorescence not emitted, while, RT-PCR is probes based and fluorescence are used to detect the gene. It can even give the results quantitatively and qualitatively and also detect the number of sample within a few time.

Commercial Assay	Parameter	Significance
LSI Vetmex Swine Influenza A (A/ H1N1/2009)	*ASFV (African Swine Flu Virus) infect domestic and wild animals.	This kit is a molecular diagnostic tool For influenza, type A virus. It is specifically detect a highly conserved region of M gene, which is specific to all influenza A virus, including the pandemic influenza A /H1N1/2009.
Real Star 3 & T Influenza RT-PCR Kit 3.0(Altona Diagnosis)	It can quantitatively detect and differentiate the influenza specific RNA (influenza A/B/ and Swine Flu (H1N1nv)	It is able to detect the seasonal influenza A and B virus as well as H1N1 strain. The probes were labeled with fluorescent reporter and quencher dyes specific for Seasonal influenza A by Cy5, Pandemic influenza A H1N1 by FAM and JOE for Internal Control.
Biomeriux (Adiavet Swine)	H1N1, H5N1 and H3N2.	It can detect influenza A and B rapid Test and also detect and differentiate B and A. Rapid test means reducing the risk of transmission sand outbreak preventing inappropriate antibiotic use and ensuring early prescription of antiviral medication for high-risk patient.
FTD (Fast Track Diagnosis) Flu/HRSV	H1N1,H3N1,H5N1,H7N1,HRV,HP IV3,HPIV4(Human parainfluenza Virus),RSVA/B(Human Respiratory Syncytial Virus) etc.	It is much cheaper and the time taken for reporting was only 29 minutes more. The low cost custom multiplex RT-PCR can be useful alternative to the costly FTD kit for rapid identification of viral etiology in resource limited settings and detect the influenza A and B virus.
Seegen's Allplex Respiratory Panel 1(Allplex RP1)	FLUAV-H1 pdm09 and FLUAV H3 respiratory.	It is a multiplex PCR for detecting 16 respiratory viruses with influenza A virus (Flu A) subtyping and the first clinical assay based on multiple detection temperatures. The All plex respiratory panel 1/2/3 which detect 16 respiratory viruses Simultaneously with influenza A subtyping represents the first clinical assay based on multiple detection temperature (MuDT), Which enables the detection of multiple targets in signal channel multiplex without melting curve analysis via RT-PCR.
Qiagen Artus INFL/ HILC/RG RT-PCR KIT	In addition of influenza A and B, the kit contains an extra reaction mix for the specific identification of Influenza A (H1N1) virus.	It is use as molecular detection kit for Real Time PCR. The kit includes a reaction mix containing all reagents and enzymes for amplification and detection of all known influenza A and B virus.

Table 1: Comparison of different commercial diagnostic kits for the molecular characterization of Swine flu.

Conclusion

During the past 30 years, molecular techniques have been under development to detect the responsible infection causing organisms to cure and treatment to the patients as fast as. The different techniques were used to detect the infectious organisms such as Culture techniques, RIDT setc assay but these all techniques takes 2 to 3 days to give confirmation to the patients and its may possible to spread out the infection in between the days of diagnosis before getting confirmation from the tests. As possible as, detection of causing organism is necessary to start the Isolation and treatment of the patients for the case of Influenza and H1N1 Swine flu. In such cases, molecular techniques like RT-PCR and different commercial kits are the most helpful diagnostic kits to detect the influenza viruses and Swine flu.

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