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REVIEW ARTICLE

H1N1 AND MOLECULAR DIAGNOSIS- RAPID AND EASE IN VIRAL AND DISEASE MONITORING

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ABSTRACT

Influenza a viruses are medically significant pathogens responsible for higher mortality and morbidity throughout the world. Swine influenza known to be caused by influenza A subtypes H1N1, H1N2, H2N3,H3N1, and H3N2, is a highly contagious and an economically important disease of pigs caused by type A influenza viruses of the family Orthomyxoviridae. Mainly H1N1, H1N2, H3N2 and H3N1 subtypes of influenza A viruses are endemic in pig populations worldwide. Pigs can also be infected by humans and avian influenza viruses and, acts as 'mixing vessels' for them, giving rise to novel reassortants. Human infections with swine flu H1N1 viruses have been earlier reported to be rare. Current review focuses on the significant approach of the usage of Molecular method utilizing Real-Time PCR in detail for the detection of type A influenza virus (H1N1 subtype) in human specimens.

INTRODUCTION:

Swine influenza (swine flu), first isolated from a pig in 1930, is a respiratory disease of pigs, caused by type A influenza virus (H1N1 subtype), which is the only type of influenza virus to have caused pandemics. Swine flu outbreaks in pigs occur regularly, causing high levels of illness and low death rates. Swine influenza viruses may circulate among swine throughout the year, but most outbreaks occur during the late fall and winter months similar to outbreaks in humans. Swine flu occasionally infects people without causing large outbreaks. Only twelve cases of swine flu were reported in the United States over the last four years (January 2005 through January 2009). None of them caused deaths. An outbreak of swine flu occurred among soldiers in Fort Dix, New Jersey, in 1976. At least four soldiers became ill with swine flu and one died; all of these patients had previously been healthy. The virus was transmitted to close contacts in a basic training environment, with limited transmission outside the basic training group. The virus is thought to have circulated for a month and disappeared. The influenza virus belongs to Orthomyxoviridae family. It has three classes: A and B which only infect humans and C which is uncommon. Its genetic material is made up of eight separate segments. The virus is enveloped with two important projections on the surface, these are haemagglutinin that binds to cell receptors in target tissues and neuraminidase that cleaves to the sialic acid in the cell wall to release the progeny viruses. Influenza A has 16 different haemagglutinins and 9 different neuraminidases. It is classified according to the types of haemagglutinin and neurominidase on its surface, e.g. H1N1, H3N2 and H5N1.Like all influenza viruses, swine flu viruses change constantly. Pigs can be infected by avian influenza and human influenza viruses as well as swine influenza viruses. When influenza viruses from different species infect pigs, the viruses can reassort (i.e. swap genes) and new viruses that are a mix of swine, human and/or avian influenza viruses can emerge. Over the years, different variations of swine flu viruses have emerged. At this time, there are four main influenza type A virus subtypes that have been isolated in pigs: H1N1, H1N2, H3N2, and H3N1. However, most of the recently isolated influenza viruses from pigs have been H1N1 viruses. The epidemic situation of A H1N1 flu arose in North America in April 2009, which rapidly expanded to three continents of Europe, Asia and Africa, with the risk ranking up to level five. Until May 13th, the flu virus of A H1N1 had spread into 33 countries and areas, with a laboratory confirmed case number of 5728, including 61 deaths. On 17th April 2009, the Center of Disease Control and Prevention (CDC), in the USA, reported Influenza A H1N1 strain with quadruple segment translocation in its RNA. On 11th June 2009 it was declared by the World Health Organization (WHO) to be a Phase 6 pandemic virus (maximum threat).This was the first declared flu pandemic in 41 years. Influenza pandemics have many effects on people, health care services and countries. The pandemic spread of influenza viruses is characterized by a high attack rate and an increased level of mortality particularly in young adults. Therefore, it

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necessary to understand influenza viruses that cause pandemics and what strategies can be used for surveillance, mitigation and control.

HISTORY:

The virus responsible for human epidemic influenza was first isolated 50 years ago by laboratory infection of ferrets with human nasal washings. This isolation was the culmination of 15 years of research to find the causative agent of the influenza pandemic of 1918, which in the space of 4 months resulted in 20 million deaths, and since when epidemic influenza has remained the most serious unconquered acute threat to human health . Global pandemics have been observed for several hundred years. The best documented pandemic occurred in **1918 (A (H1N1), Spanish flu**). It was estimated to have infected 50% of the world's population, with an estimated mortality of 40–50 million (mortality rate of 2–2.5%). The attack and mortality rates were highest among healthy adults (20–40 years old). The second was in **1957 (A(H2N2), Asian flu)** which affected around 40–50% of people during two waves, with a mortality rate of 1 in 4000 and the total death toll probably exceeding 1 million. The third was in **1968 (A (H3N2), Hong Kong flu)** with similar morbidity and mortality to Asian flu. Aspirin use which is known to cause hyperventilation and pulmonary oedema in high doses was the major factor in the high death rate from Spanish flu. Other possible factors could be the unavailability of antibiotics which were not yet discovered to treat bacterial super infection; primitive infection control practices and the destruction of health care facilities as a result of World War I. On April 15–17, 2009, the Centres for Disease Control and Prevention (CDC) confirmed the first two cases of human infection with the pandemic H1N1 virus in San Diego, California.By August 2009, the cumulative number of infections in the United States alone was estimated to be at least 1 million. However, there were only 556 confirmed deaths, i.e. the mortality rate was only 0.056 %. The outbreak of swine influenza A (H1N1) evolved so rapidly that as on 29 April 2009, nine countries officially reported with confirmed cases of swine influenza A/H1N1 infection. Of these, Mexico, United State, Austria, Canada, Germany, Israel, New Zealand, Spain and the United Kingdom have reported laboratory confirmed human cases and deaths due to rapidly progressive pneumonia, respiratory failure and acute respiratory distress syndrome (ARDS). World Health Organization (WHO) declared ever high stages on its "pandemic" scale-alert 6, designating the Influenza H1N1 2009 a potential threat to worldwide health and declared the outbreak as Public Health Emergency of International Concern (PHEIC). Then in India total confirmed cases and total deaths crossed to a level were the threat of a full blown epidemic is very real.

Types of influenza Virus; Nucleoprotein and matrix are used to classify influenza viruses as Types A, B and C. **Influenza Type A:** Influenza type A viruses can infect people, birds, pigs, horses, seals, whales, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes based on two proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA). There are 15 different HA subtypes and 9 different NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses. Subtypes of influenza A virus are named according to their HA and NA surface proteins. For example, an “H7N2 virus” designates influenza A subtype that has an HA 7 protein and an NA 2 protein. Similarly an “H5N1” virus has an HA 5 protein and an NA 1 protein. **Influenza Type B:** Influenza B viruses are normally found only in humans. Unlike influenza A viruses, these viruses are not classified according to subtype. Although influenza type B viruses can cause human epidemics, they have not caused pandemics. **Influenza Type C**: Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. These viruses are not classified according to subtype.

ENTRY OF H1N1 IN INDIA AND ITS CONSEQUENCES:

The first case of the flu in India was found on the Hyderabad airport on 13 May, when a man travelling from US to India was found H1N1 positive. Subsequently, more confirmed cases were reported and as the rate of transmission of the flu increased in the beginning of August, with the first death due to swine flu in India in Pune, panic began to spread. As of 24 May 2010, 10193 cases of swine flu have been confirmed with 1035 deaths. For early diagnosis and detection of H1N1virus, PCR testing is highly sensitive (lower limit of detection, 1–10 infectious units). Real-time PCR is the test of choice for influenza A H1N1 2009. It is more rapid and sensitive than cell culture as the continuous evolution of influenza genomes together with reassortment events pose challenges to the effective monitoring of influenza viruses in circulation. Swine flu, also called Hog or Pig Flu, is an infection caused by any one of the several types of Swine influenza virus (SIV) which is common throughout pig population worldwide. The term "influenza" derived from Italian word" influence" was coined ***in 1357 AD*** as the disease was thought to be caused

by influence of stars. Influenza pandemics are believed to have occurred at unpredictable intervals for many centuries .Influenza as a disease of pigs was first recognized during the Spanish influenza pandemic of 1918–1919. Veterinarian J. S. Koen was the first to describe the illness, observing frequent outbreaks of influenza in families followed immediately by illness in their swine herds, and vice versa . Influenza virus was first isolated from pigs in 1930 by Shope and Lewis, with the virus isolated from humans several years later. The first isolation of a swine influenza virus from a human occurred in 1974, confirming speculation that swine origin influenza viruses could infect humans .

STRUCTURE OF SWINE INFLUENZA (H1N1) VIRUS:

Influenza viruses are enveloped, segmented, single-stranded, negative-sense RNA viruses belonging to the Orthomyxoviridae family. Influenza viruses contain eight RNA genes that code for eight proteins – internal and external structural proteins, RNA polymerase, and nonstructural proteins.Each gene segment contains a coding region that encodes one or two viral proteins; three segments (1, 2 and 3) encode proteins that form the viral polymerase complex: polymerase basic protein 2 (PB2), PB1 and polymerase acidic protein (PA), respectively. Two segments (4 and 6) encode surface envelope glycoproteins that function as viral antigens, HA and NA, respectively. Segment 5 encodes NP. Segment 7 encodes two proteins, the matrix protein M1 and M2. The smallest segment 8 encodes two non-structural proteins NS1 and NS2. Three phylogenetically and antigenically distinct viral subtypes, A, B and C, are circulating globally among human populations, and subtype A influenza viruses have exhibited the greatest genetic diversity, infected the widest range of host species, and caused the vast majority of severe disease in humans. The influenza A viruses are further subdivided by antigenic characterization of the surface glycoproteins HA and NA; so far, 16 HA subtypes (H1-H16) and nine NA subtypes (N1-N9) are known.These two proteins are involved in cell attachment and release from cells, and are also major targets for the immune response.2,20,131 Wild birds carry most of the known hemagglutinin and neuraminidase antigens, but some, such as H14 and H15, are uncommon and seem to occur only in limited geographic areas. Only limited subtypes are found in each species of mammal. Influenza A viruses are also classified into strains. Strains of influenza viruses are described by their type, host, place of first isolation, strain number (if any), year of isolation, and antigenic subtype.1,3 [e.g., the prototype strain of the H7N7 subtype of equine influenza virus, first isolated in Czechoslovakia in 1956, is A/eq/Prague/56 (H7N7).] For human strains, the host is omitted.

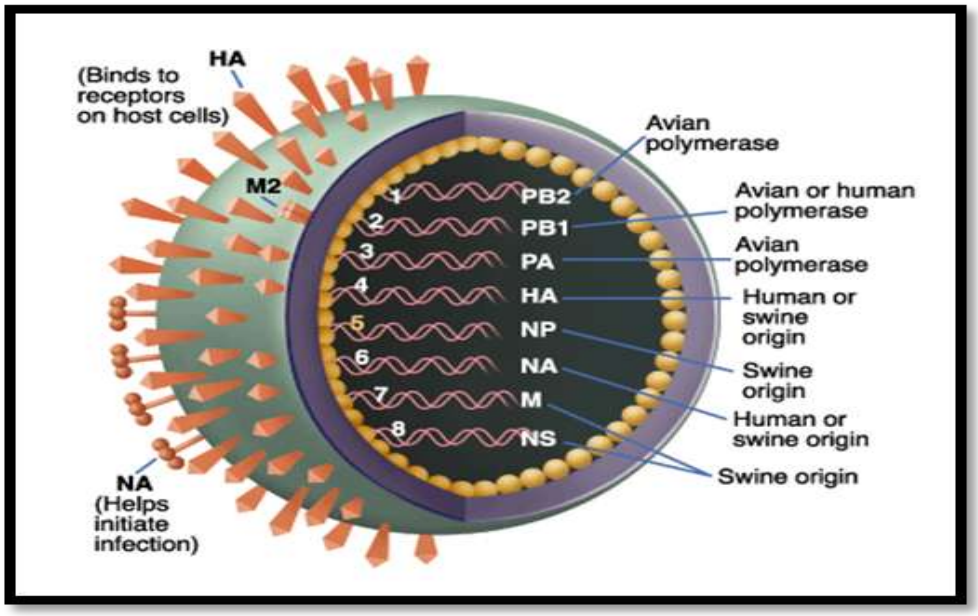

Figure 1: Picture depicting structure of Influenza virus

EVOLUTION OF INFLUENZA VIRUS:

Influenza A virus evolution is considered to be predominantly driven by two mechanisms known as **antigenic drift** and **antigenic shift**. Influenza viruses are changing by antigenic drift all the time, but antigenic shift happens only occasionally. Influenza type A viruses undergo both kinds of changes; influenza type B viruses change only by the more gradual process of antigenic drift.

Antigenic Drift: Antigenic drift occurs by random mutation and single amino acid substitution in the HA and NA proteins during viral replication. The change is gradual and part of the normal drift seen with SIV. For the HA gene of

influenza viruses, a mutation occurs at the rate of one mutation in every 100 replicated genes. This rate is sufficiently high enough to create several antigenic variants each year. As in all RNA viruses, mutations in influenza occur frequently because the virus' RNA polymerase has no proofreading mechanism, providing a strong source of mutations. Mutations in the surface proteins allow the virus to elude some host immunity.

Antigenic shift: Antigenic shift is an abrupt, major change in the influenza A viruses by which two or more different strains of a virus, or strains of two or more different viruses, combine to form a new subtype having a mixture of the surface antigens of the two or more original strain resulting in a new influenza virus that can infect humans and has a hemagglutinin protein or hemagglutinin and neuraminidase protein combination that has not been seen in humans for many years. When two different strains of influenza infect the same cell simultaneously, their protein capsids and lipid envelopes are removed, exposing their RNA, which is then transcribed to mRNA. The host cell then forms new viruses that combine their antigens; for example, H3N2 and H5N1 can form H5N2 this way.

TRANSMISSION:

Transmission between pigs: Influenza is quite common in pigs, with about half of breeding pigs having been exposed to the virus in the US. Antibodies to the virus are also common in pigs in other countries. The main route of transmission is through direct contact between infected and uninfected animals. These close contacts are particularly common during animal transport. Intensive farming may also increase the risk of transmission, as the pigs are raised in very close proximity to each other. The direct transfer of the virus probably occurs either by pigs touching noses, or through dried mucus. Airborne transmissions through the aerosols produced by pigs coughing or sneezing are also an important means of infection. The virus usually spreads quickly through a herd, infecting all the pigs within just a few days. Transmission may also occur through wild animals, such as wild boar, which can spread the disease between farms. **Transmission to humans:** People who work with poultry and swine, especially people with intense exposures, are at increased risk of zoonotic infection with influenza virus endemic in these animals, and constitute a population of human hosts in which zoonosis and reassortment can co-occur. Vaccination of these workers against influenza and surveillance for new influenza strains among this population may therefore be an important public health measure. Transmission of influenza from swine to humans who work with swine was documented in a small surveillance study performed in 2004 at the University of Iowa. This study among others forms the basis of a recommendation that people whose jobs involve handling poultry and swine be the focus of increased public health surveillance. Other professions at particular risk of infection are veterinarians and meat processing workers, although the risk of infection for both of these groups is lower than that of farm workers.

PATHOGENESIS & REPLICATION:

The predominant way in which influenza is transmitted is from person to person by aerosols and droplets. Influenza then enters the host through the respiratory tract. In a human lung there are about 300 million terminal sacs, called alveoli, Small droplets with a diameter of approximately 1 to 4 µm precipitate in the small airways. Much larger particles are either not able to enter the respiratory system or are deposited in the upper respiratory tract. The respiratory tract is covered with a mucociliary layer consisting of ciliated cells, mucus-secreting cells and glands. Foreign particles in the nasal cavity or upper respiratory tract are trapped in mucus, carried back to the throat, and swallowed. From the lower respiratory tract foreign particles are brought up by the ciliary action of epithelial cells. In the alveoli that lack cilia or mucus, macrophages are responsible for destroying particles.

BINDING TO THE HOST CELLS:

In influenza infection, the receptor binding site of viral hemagglutinin (HA) is required for binding to galactose bound sialic acid on the surface of host cells.Certain areas of the binding site of HA are highly conserved between subtypes of the influenza virus.

Hosts may prevent the attachment by several mechanisms: (1) specific immune response and secretion of specific IgA antibodies, (2) unspecific mechanisms, such as mucociliary clearance or production of mucoproteins that able to bind to viral hemagglutinin, and (3) genetic diversification of the host receptor (sialic acid), which is highly conserved in the same species, but differs between avian and human receptors.The virulence of the influenza virus depends on the compatibility of neuraminidase with hemagglutinin. A virulent virus which has undergone mutations in the hemagglutinin needs compensatory mutations in the neuraminidase to maintain its virulence.Once the cell membrane and the virus have been closely juxtaposed by virus-receptor interaction, the complex is endocytosed. Importing H+ ions into the late endocytic vesicles as a physiologic event then acidifies the interior. Upon acidification, the viral HA undergoes a conformational rearrangement that produces a fusiogenic protein. The loop region of the HA becomes a coiled coil

eventually bringing the viral and endosomal membranes closer so that fusion can occur. To allow release of viral RNA into the cytoplasm, the H+ ions in the acidic endosome are pumped into the virion interior by the M2 ion channel. As a result, viral RNA dissociates from M1 by disrupting the low pH-sensitive interaction between the M1 and ribonuclein complex after fusion of the viral and endosomal membranes. The viral RNA is then imported in an ATP-dependent manner into the nucleus for transcription and translation.Once influenza has efficiently infected respiratory epithelial cells, replication occurs within hours and numerous virions are produced. Infectious particles are preferentially released from the apical plasma membrane of epithelial cells into the airways by a process called budding. This favors the swift spread of the virus within the lungs due to the rapid infection of neighboring cells. Alterations in the HA cleavage site by naturally occurring mutants can dramatically influence the tropism and pathogenicity of influenza. As a result, it can be recognized by other cellular proteases.. This leads to higher local concentrations of this ubiquitous protease precursor and thus to increased cleavage of the HA.

DIAGNOSIS:

Influenza A H1N1 2009 virus can be detected in respiratory specimens by different tests. These tests differ in their sensitivity, specificity and ability to distinguish between influenza A subtypes (e.g. 2009 H1N1 versus seasonal H1N1 versus seasonal H3N2 viruses).

Rapid influenza diagnostic tests (RIDTs) have variable sensitivities and specificities, some expert shaving reported sensitivity of 47%, and specificity of 86%.48 Others have reported sensitivity of 51%, and specificity of 99%.

Direct immunofluorescence (DIF) has variable sensitivities (47–93%), but high specificity ≥96 %.Some reports claim that the DIF has a sensitivity of 93%, specificity of 97%, positive predictive value of 95% and negative predictive value of 96%.**Viral culture** is considered as gold standard for influenza virus testing; however, it is only 88.9% sensitive for Influenza A H1N1 2009.Therefore, a negative viral culture does not exclude infection with influenza A H1N1 2009.. Some researchers have described detection of the virus using microarray techniques.**PCR testing** is highly sensitive (lower limit of detection, 1–10 infectious units).

Real Time-PCR for Diagnosis of H1N1is the test of choice for influenza A H1N1 2009.It is more rapid and sensitive than cell culture. However, PCR is expensive and labour intensive; therefore, it is impractical to investigate all affected patients because of the large number of people infected.The most Powerful DNA amplification technology known as on date in molecular biology first described by Higuchi in 1992.

Sample collection for H1N1: Samples should be taken from the nasopharynx (a nasopharyngeal swab), nasopharyngeal aspirates, throat swabs and Trans bronchial aspirates. Swab specimens should be collected using swabs with a synthetic tip (e.g. polyester or Dacron®), but not calcium alginate or cotton tips; the shaft should be made of aluminum or plastic, but not of wood. Specimens should be placed into sterile viral transport media.

Materials for specimen collection include transport Media, which can be commercial viral transport media or In house viral transport medium. Preparation of In-house transportation media: **A) Medium 199 :**Tissue culture medium 199 contains 0.5% bovine albumin fraction V, Penicillin G (2 X 106 U/liter), Streptomycin 200 mg/liter, polymyxin (2 x 106 U/liter), gentamicin (250 mg/liter), nystatin (0.5 X 106 U/ liter). Ofloxacin HCl (60 mg/liter) and sulfamethoxazole (0.2 g/ liter). **B) Broth media:** 10g veal infusion broth, 2g of BSA fraction V add it to 400ml sterile distilled water Penicillin G (2 X 106 U/liter), Streptomycin 200 mg/liter, Polymyxin (2 x 106 U/liter), Gentamicin (250 mg/liter), nystatin (0.5 X 106 U/liter), Ofloxacin HCl (60 mg/liter) and Sulfamethoxazole (0.2 g/ liter). Sterilize by filtration and distribute in 1.0ml - 2.0ml volumes in screw capped tubes clubbed with sterile dacron swabs. Calcium alginate is not accepted for the collection of viral specimens.

Upper respiratory tract specimens: Method of collecting a throat swab, Hold the tongue down with the depressor. Use a strong light source to locate areas of inflammation in the posterior pharynx and the tonsillar region of the throat behind the uvula. Rub the area back and forth with the swab. Withdraw the swab without touching cheeks, teeth or gums and insert into a screw-cap vial containing viral transport medium. Break off the top part of the stick without touching the tube and tighten the screw cap firmly. Label the specimen containers with patient's name type of specimen and date of collection. Complete the laboratory request form.

Method of collecting Nasopharyngeal Swabs (per-nasal and post nasal swab): Seat the patient comfortable, tilt the head back. Insert a flexible swab beneath the inferior turbinate of either nostril or leave in place for a few seconds and move the swab upwards into the nasopharyngeal space. Rotate the swab on the nasopharyngeal membrane a few times; slowly withdraw

with a rotating motion against the mucosal surface of the nostril. Remove the swab carefully and insert it into a screw-cap tube containing transport medium. Repeat the procedure in the other nostril using a new sterile swab the tip of each swab is put into a vial containing 2-3 ml of viral transport media (VTM), and the applicator stick is broken off. Label vial with patient's name, specimen type & date of collection; complete lab request form. **Aspirates:** Nasopharyngeal secretions are aspirated through catheter connected to a mucus trap and fitted to a vacuum source. The nasal aspirates are collected by introducing a few ml of saline into the nose with a syringe fitted with affine tubing or catheter. The catheter is inserted into a nostril parallel to the palate. Then the vacuum is applied and the catheter is slowly withdrawn with a rotation motion. Mucus from the other nostril is collected with the same catheter in a similar manner. After mucus has been collected from both nostrils, the catheter is flushed into a screw cap vial with 3 ml viral transport media. Label the vial with patient's name type of specimen and date of collection**.** Complete the laboratory request form.

SHIPMENT OF SPECIMENS:

The specimen(s) must be shipped immediately, if delay is more than 4 hours of collection the specimen should be refrigerated and send with ice packs. Wrap the primary container (the container in which the specimen is enclosed such as a vial) with parafilm or sealing tape around the lid. The container must then be wrapped with enough absorbent material to absorb all of the fluid in the primary container. (Note: If using paper towels as absorbent material, use at least one paper towel for each 1.5 ml of fluid). Additional absorbent should be placed around the container to prevent breakage during transport. Place the specimen primary container and absorbent wrapping into a sealable plastic bag (the specimen + absorbant + plastic bag). Place the plastic bag (the specimen + absorbant + plastic bag) into a secondary close container.

IMPORTANT INSTRUCTION ON THE LABORATORY REQUEST FORM:

Patient demographics, clinical signs and symptoms, date of onset of illness and date of collection of specimen, type of specimen, travel history and/or contact of known case. **In Land Transportation of Diagnostic Specimens:** If the sentinel site is located away from the National Laboratory. Place the plastic bag (the specimen + absorbent + plastic bag) into a secure the secondary safety container. Place the sample container in a cooler with ice packs to ensure specimen integrity in hot weather during transit from the sentinel site to the national laboratory, Send the specimen and the Laboratory Request Form with the a previously trained carrier or driver dedicated to the transportation of specimens.

Overseas Transportation of Diagnostic Specimens: Place the plastic bag (the specimen + absorbent + plastic bag) into a secure the secondary safety container is showed in the figure below with the laboratory request form. Place the sample container in cardboard container with ice packs. Communicate with your national public health authority before referring samples to Satellite and CAREC laboratories. Notify the satellite and CAREC laboratory of the shipment of clinical specimens. Submit specimens to your Satellite Laboratory and CAREC Laboratory Division, through the National Laboratory according CAREC guidelines and the IATA regulations "Diagnostic specimens" UN 3373.

Transportation of specimens: Specimens should be sent as "diagnostic specimens" in accordance with the International Air Transport Association dangerous goods regulations.

H1NI and Molecular Diagnosis

Viral RNA is isolated after sample reaches the laboratory. H1N1 virus RNA Isolation QIAamp Viral Mini Kit, or Rneasy Mini Kit (QIAGEN) approved for IVD by FDAcan only be used for RNA extraction.

Master Mix preparation for H1N1: Prepare the pre mix (from Lab India/Applied Biosystem, RT-PCR specific for the pandemic H1N1 2009 virus HA gene: a new probe is used NIID-swH1 Probe2) as shown in table 1.

Table 1: Composition of pre-mix for the detection of H1N1 by Real-Time PCR.

Sr. No.	Component/s	Final vol. for 1X
1.	RT PCR buffer Mix	12.5 µl
2.	Enzyme Mix	1.0 µl
3.	Nuclease Free H2O	6.0 µl
4.	Assay Mix(includes primers and probes for gene; RNase P, Inf A, Swinf, SwiH1)	0.5 µl
5	**Total**	**20.0 µl**

The above process is for 1 reaction but to perform for multiple samples, multiply the no. of samples accordingly.

PRIMER AND PROBE SETS:

Primers and probes	Sequence (5' >3')	Working Concentration
Inf A Forward	GAC CRA TCC TGT CAC CTC TGA C	40 µM
Inf A Reverse	AGG GCA TTY TGG ACA AAK CGT CTA	40 µM
Inf A Probe	TGC AGT CCT CGC TCA CTG GGC ACG	10 µM
SW Inf A Forward	GCA CGG TCA GCA CTT ATY CTR AG	40 µM
SW Inf A Reverse	GTG RGC TGG GTT TTC ATT TGG TC	40 µM
SW Inf A Probe	CYA CTG CAA GCC CA"T' ACA CAC AAG CAG GCA	10 µM
SW H1 Forward	GTG CTA TAA ACA CCA GCC TYC CA	40 µM
SW H1 Reverse	CGG GAT ATT CCT TAA TCC TGT RGC	40 µM
SW H1 Probe	CA GAA TAT ACA "T"CC RGT CAC AAT TGG ARA A	10 µM
Rnase P Forward	AGA TTT GGA CCT GCG AGC G	40 µM
Rnase P Reverse	GAG CGG CTG TCT CCA CAA GT	40 µM
Rnase P Probe	TTC TGA CCT GAA GGC TCT GCG CG	10 µM

Table 2: Primers and probes for Real Time PCR for the molecular diagnosis of H1N1.

CLINICAL SIGNIFICANCE OF THE RESULTS:

The Real Time PCR detection chemistry is based on the FRET (fluorescence resonance energy transfer), with the utilization of the TaqMan probe. In the particular assay, the usage of the four molecular targets which includes; influenza A, Swine influenza, Swine HINI and a housekeeping gene called Rnase P, acting as an internal control. The assay can simultaneously amplify seasonal influenza as well as swine flu with in the same run in a very short span of time (3-4 hours). The pandemic influenza A H1N1 is currently the most prevalent influenza virus. Most of the cases are mild, but there are high incidences in children and young adults. The presentation and complications are similar to those caused by seasonal influenza strains, but the mortality rate to date seems to be lower compared to seasonal strains. Vaccines and antivirals are available that can provide protection from infection. However, new viral strains emerge continuously because of the plasticity of the influenza genome, which necessitates annual reformulation of vaccine antigens, and resistance to antivirals can appear rapidly and become entrenched in circulating virus populations. In this study, a real-time reverse transcriptase PCR (RT-PCR) assay based on the hemagglutinin gene was developed that discriminates the novel H1N1 from swine influenza virus. This sensitive and specific real-time RT-PCR assay will contribute to the early diagnosis and control of the emerging H1N1 influenza pandemic.

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CONFLICT OF INTEREST: NONE:

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