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The Laboratorian SM

H1N1 Influenza in Pregnancy

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Influenza viruses are divided into three serological types (A, B and C) according to the antigenicity of conserved viral proteins. Influenza viruses B and C are usually human specific, while influenza A virus is preferentially endemic in water birds and shore birds, which usually do not fall ill from this infection. Two Influenza A virally encoded proteins, hemagglutinin (H) and neuraminidase (N) mediate viral infectivity and maturation. Influenza A viruses are divided into 16 H and 9 N serological subtypes. To date, 105 influenza A virus subtypes have been discovered (1).

Influenza A is currently the greatest pandemic disease threat to humankind. Influenza A is unique among the major pandemic threats in that it could potentially infect 30% of the world's population within a matter of months. With a conservative mortality rate of 2%, it would result in approximately 135 million deaths worldwide within the first year of the outbreak. This is about 4 times the total mortality attributed to HIV-1 in the last 30 years (2).

Influenza's endemic reservoir is in aquatic wildfowl, many of which are migratory. Once a novel strain of influenza has crossed the species barrier from birds into a mammalian host, it may persist in that new host species for many decades. It is also capable of transmission between mammals. The Influenza A H1N1 2009 outbreak is now known to have originated in pigs (3).

Epidemiology:
In humans, three subtypes of influenza A virus (H1N1, H2N2, H3N2) verifiably caused pandemics of high morbidity and mortality (Table 1) (4). The 1918/1919 pandemic, known as the Spanish flu, left one-third of the world's population ill and caused at least 50 million deaths worldwide (5). The most recent influenza pandemics, including Asian influenza (H2N2; 1957-1963; about 2-4 million persons killed) and Hong Kong influenza (H3N2; 1968-1970; about 1-2 million persons killed), were less aggressive.

2009 Swine-origin Influenza A (H1N1) Virus Outbreak in Humans:
Currently, the World Health Organization (WHO) level of pandemic alert is currently at level 5 of 6 possible levels, indicating that a pandemic is considered to be imminent (http://www.who.int/csr/disease/swineflu/en/index.html). The first death from H1N1 occurred on April 13, 2009, when a diabetic woman from Oaxaca, Mexico died from respiratory complications. By May 13, 2009 33 countries had officially reported 5,722 cases of H1N1 infection resulting in 61 deaths. The United States had reported 3,009 laboratory confirmed human cases, including three deaths (http://www.who.int/csr/don/2009_05_13/en/index.html). As of July 24, the CDC had reported 43,771 confirmed cases of novel H1N1 infection in the United States with 302 confirmed deaths (6).

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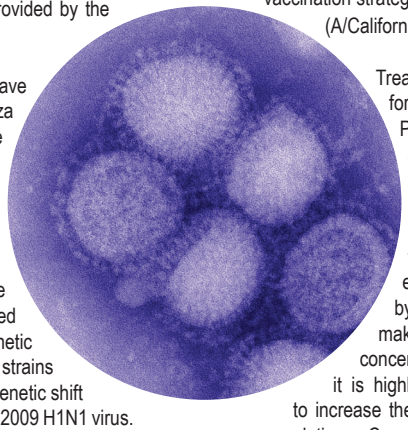
H1N1 Influenza

Author: Kathryn Iacono, Ph.D.

During the spring of 2009 a novel strain of H1N1 influenza was detected within the United States that was elevated to a pandemic status by the World Health Organization in early June. The designation as "swine" flu came from preliminary laboratory analyses that identified similarities at the genetic level between the circulating virus and swine strains that have been in circulation among pigs in North America for several years. Further analyses of the viral genome revealed the circulating strain as a whole to be completely unique from previously circulating strains. This novel influenza strain came to be as a result of a quadruple viral reassortment, whereby genetic elements of Asian and European circulating swine flus combined with genetic elements from avian and human strains. This mixture of genetic components combined to form an antigenically distinct strain of influenza that poses a significant health risk throughout the world due to the lack of immune memory. The information provided in this article is an encapsulation of the information provided by the CDC on their website (http://www.cdc.gov/flu/swineflu/).



The emergence of recombinant strains, against which people have little or no immunity, increases the likelihood of another influenza pandemic, an infectious epidemic affecting all continents. The worst influenza pandemic to date occurred in 1918 and resulted in the death of 50 million people worldwide. While pandemics of the 1918 proportion typically do not happen frequently, occurring approximately every 50 years, the ability of the influenzas to undergo genetic reassortment increases the likelihood of developing another highly lethal strain. The influenza viruses are more prone to such events because their genomes are comprised of RNA segments, which easily allows for swapping of genetic elements during co-infections. The process by which new viral strains are generated as a result of segment swapping is referred to as genetic shift and it is this process that directly led to the generation of the novel 2009 H1N1 virus.

In general, H1N1 viruses that have infected humans in the past have proven to be less infectious than the H3N2 strains that have dominated recent influenza seasons. These previous H1N1 exposures brought about mild cases of the flu but left individuals more prone to secondary respiratory infections, like pneumonia. This is generally true when considering the 2009 H1N1 virus despite the fact that this strain is an antigenically distinct virus and no previous information upon which to establish guidelines exists. Based upon the infectious cases observed during the spring outbreak, the CDC states that most people infected with H1N1 will recover without complication. However, these same spring infections also identified at-risk populations to include pregnant women, individuals over the age of sixty-five, individuals with predisposing chronic conditions (asthma, cardiovascular disease, diabetes) and the immunocompromised. Prevention is still the best means of limiting transmission and, as such individuals are encouraged to improve both their personal and environmental hygiene, limit their exposure to infected individuals and get vaccinated. Adequate vaccination coverage has been shown to markedly decrease the infectivity rate of seasonal influenza and, at this time, there is no reason to believe the 2009 H1N1 strain will respond any differently. There are currently two types of immunizations available: inactivated influenza vaccine administered by shot and the live, attenuated influenza vaccine (LAIV) administered intranasally. Both vaccination strategies are monovalent, having been generated from a circulating H1N1 strain (A/California/7/09) isolated in the spring.

Treatment should be limited to confirmed cases within the at-risk populations for now. The CDC defines a confirmed case as one that has used either a PCR-based or viral culture methodology to identify the H1N1 virus. Rapid antigen assays, due to their inability to subspeciate among the influenza A strains are not confirmatory. Also, these assays cannot be used to regain admittance to communal organizations that have enacted policies banning infected individuals in an effort to limit infection. Two methods of antiviral therapy, oseltamivir (Tamiflu) and zanamivir (Relenza) have been proven effective against H1N1. These drugs work to shorten the duration of illness by approximately twenty-four hours, which in some at-risk populations could make a dramatic difference as far as hospitalization length and mortality are concerned. While prophylactic treatment with these agents would be tempting, it is highly discouraged. The inappropriate administration of these drugs works to increase the levels of drug resistance which ultimately limits their efficacy within all populations. Currently, very low levels of oseltamivir resistance have been identified in association with H1N1 viruses, but as Table 2 depicts in the instance of the adamantine class of antivirals used to combat H3N2 infections, resistance can mount over a surprisingly short period of time rendering the drug useless. Medical Diagnostic Laboratories offers a PCR-based molecular assay that specifically identifies the 2009 H1N1 Swine Flu that includes reflex analysis of the viral sequence to screen for oseltamivir (Tamiflu) resistant strains for all positive specimens at no additional charge. Providing this information allows physicians and patients to be better informed and to take the precautionary measures to limit its spread.

Journal Watch

CDC. (2009). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic Influenza A (H1N1)—United States, May-August 2009. *MMWR* 2009;58 (1071-74).

Seventy-seven postmortem tissue specimens obtained from individuals who succumbed from their 2009 H1N1 infections between May 1 and August 20 were analyzed to determine the role of bacterial coinfections during this pandemic. In previous pandemics such analyses have revealed most Influenza A fatalities were associated with concurrent bacterial pneumonia infections. This report set out to demonstrate similar linked causality that has heretofore not been published with respect to H1N1. Multiple experimental analyses utilizing both immunohistochemical and molecular techniques were used to determine the presence and identification of the coinfecting pathogens in these specimens. Bacteria were identified in 22 of the 77 cases (29%) and included: 10 cases with *S. pneumoniae*, 6 cases with *S. pyogenes*, 7 cases with *S. aureus*, 2 with *S. mitis* and 1 with *H. influenzae*. Four cases were found to involve multiple pathogens. This is the first report since the inception of the H1N1 pandemic that demonstrates a link between viral infection and secondary bacterial infections of the respiratory tract. Limited clinical information was available for the majority of the cases analyzed, but underlying medical conditions known to increase the risk of influenza-associated complications were associated with some cases. Based on these findings, the CDC strongly urges physicians to consider the possibility of bacterial coinfections and to include pneumococcal vaccine for all individuals fulfilling the Advisory Committee on Immunization Practices recommendations when administering the seasonal and H1N1 vaccinations within at-risk populations.

Saleeby E, Chapman J, Morse J, Bryant A. (2009). H1N1 influenza in pregnancy. *Obstet and Gynecol.* **114(4)**:885-891.

This article presents two case studies of H1N1-infected pregnant women, one who recovered from her infection while the other succumbed, and offers guidelines to be considered when dealing with this high-risk segment of the population. Because pregnant women have proven to be a highly susceptible group during the 2009 pandemic they require special consideration with respect to diagnosis, treatment and follow-up care. Due to the demands placed upon primary care physicians as a result of the influenza season, it has become common practice to triage patients by telephone, a practice that should not be used routinely with pregnant individuals. In their experience, the serial monitoring of pulse oximetry as an evaluating criteria alongside vital signs was efficacious. The authors also recommend influenza testing be performed on any pregnant woman presenting with a fever and/or sore throat during and treatment be initiated on for those having symptoms consistent with influenza-like illness flu season. A final recommendation concerns the level of follow-up care for these individuals. Because H1N1 infections have the ability to worsen fairly rapidly within this population, physicians are urged to contact those individuals receiving treatment within the first twenty-four hours of initiation and to continue to monitor their progression so long as symptoms remain.

Karre T, Maguire HF, Graepler A, Weed D, Wilson ML. (2009). Comparison of Becton Dickinson Directigen EZ Flu A+B test against the CDC RT-PCR assay for the detection of pandemic influenza A/H1N1 virus. *J Clin Micro*. Published on-line ahead of print on November 4, 2009.

This study evaluates the performance of the Directigen EZ Flu A+B rapid immunodiagnostic test to the CDC-developed RT-PCR assay that was given Emergency Use Authorization by the FDA in detecting the 2009 pandemic H1N1 influenza virus. A total of 231 nasopharyngeal wash specimen (no swabs were analyzed) were collected between August and October 2009, six of which were determined to be seasonal influenza by RT-PCR and as such excluded from the analysis. All samples were submitted to the Clinical Microbiology Laboratory at Denver Health Medical Center for analysis with the Directigen assay where aliquot were taken for submission to the Laboratory Services Division of the Colorado Department of Health and Environment for analysis with the CDC-developed H1N1 RT-PCR assay. Of the 225 total specimen analyzed, 80 were determined to be positive and 145 negative by the RT-PCR assay. The agreement between the two methodologies was reported to be 79.5%. These findings conflict with most of the previous reports that demonstrated poor performance characteristics associated with the rapid antigen assays. However, these results recapitulate previous findings that demonstrated a high degree of specificity for these rapid assays but low diagnostic value due to their inability to properly speciate among the influenza viral strains. In this regard, the authors suggest rapid antigen assays may be useful as initial screens that would identify influenza positive patients that should be evaluated by a more sensitive method like the CDC-developed RT-PCR diagnostic assay.

Charlier C, Enouf F, Lanternier F, Grandadam M, Amazzough K, Blanche S, Lortholary O, van der Werf S. (2009). Kinetic of nasopharyngeal shedding of novel swine-like influenza A (H1N1) virus in an immunocompetent adult under oseltamivir therapy. *Clin Microbiol Infect.* Published on-line ahead of print July 22, 2009.

This case report represents the first kinetic analysis of 2009 H1N1 influenza virus shedding during concurrent antiviral treatment. A French tourist traveling through Mexico during the early stages of the H1N1 pandemic was hospitalized with a mild fever, dyspnea, cough and myalgia upon his return to France. Influenza A positivity was determined following RT-PCR analysis of an obtained nasopharyngeal swab and further molecular analyses revealed the influenza strain to be H1N1. Antiviral therapy in the form of oseltamivir (75 mg X 2/d) was initiated 48 hours after the onset of symptoms. Nasopharyngeal swabs were collected and analyzed on a daily basis following oseltamivir administration. H1N1 was detectable for the first two days of antiviral treatment but was undetectable by day 3. Therefore, the kinetics of H1N1 viral shedding appear to mirror that of seasonal H3N2 influenza A and influenza B viruses. This preliminary report suggests that the prompt initiation of antiviral therapy will reduce transmission rates within the general populace by increasing viral clearance within affected individuals.

Alexander J, Bilsel P, del Guerico MF, Stewart S, Marinkovic-Petrovic A, Southwood S, Crimi C, Vang L, Walker L, Ishioka G, Chitnis V, Sette A, Assarsson E, Hannaman D, Botten J, Newman MJ. (2009). Universal influenza DNA vaccine encoding conserved $CD4^+$ T cell epitopes protects against lethal viral challenge in HLA-DR transgenic mice. *Vaccine*. Published on-line ahead of print November 3, 2009.

The aim of this study was to design a universal influenza A vaccine that would work irrespective of the annually changing circulating viral strains. To this end, the sequences of a number of influenza A strains, including currently circulating H1N1 and H3N2 influenza A strains were analyzed along with three strains associated with past pandemics and six zoonotic strains that may serve as the reservoir for future pandemics were analyzed to identify conserved regions among them. The amino acid sequences of these regions were then evaluated for the presence of regions that could serve as antigenic epitopes through association with HLA-DR molecules. From this initial screen, twenty candidates were selected for further evaluation based on their conservation among the various viral strains, their predicted coverage of diverse ethnic groups and ability to initiate memory immune responses upon subsequent influenza infection in HLA-DR4 transgenic mice. Challenging the mice with a lethal strain of influenza revealed a protective effect for some epitopes. However, due to the pleiomorphic nature of the HLA region, no single peptide demonstrated the broad spectrum protection that was anticipated. The authors suggest efficacy could be attained through the administration of a vaccine designed against several of the promising peptides and argue that these findings, though preliminary, may one day take the guessing game out of annual influenza vaccine design strategies.

H1N1 Influenza in Pregnancy References:

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HUMIGEN: Recent Publications

Genetic Immunology Laboratory

Peer-Reviewed Papers: Recent Publications:

1. **Dai J, Megjugorac N, Gallagher GE, and Gallagher G.** IFN-l1 (Il-29) inhibits GATA3 expression and suppresses Th2 responses in human naïve and memory T cells. *Blood,* 2009 June, **113(23):** 5829-38.
2. **Gallagher GE, Gallagher G, and Nick Megjugorac** Modulation of human plasmacytoid DC function by IFN-l1 (IL-29). *Journal of Leukocyte Biology, Sep. 16, Epub ahead of print.*

Genomic Immunoepidemiology Laboratory

1. **Do T, Ucisik-Akkaya E, Davis C, Morrison B and Dorak MT.** TP53 R72P and MDM2 SNP309 Polymorphisms in Modification of Childhood Acute Lymphblastic Leukemia Susceptibility, *Cancer Genetics and Cytogenetics*, 2009, **195:** 31-36.
2. **Davis C and Dorak MT.** An Extensive Analysis of the Hereditary Hemochromatosis Gene HFE and Neighboring Histone Genes, *Annals of Hematology, Oct. 6. Epub ahead of print.*
3. **Do T and Dorak MT.** An Intronic Polymorphism of IRF4 Gene Influences Gene Transcription in vitro and Shows a Risk Association with Childhood ALL in Males. *BBA Molecular Basis of Disease, Nov. 5. Epub ahead of print.*

Recently Presented Abstracts

1. **Do T, Davis C, Ucisik-Akkaya, E, Morrison B, and Dorak MT**. November 2-6, 2009. Molecular Mechanism of Sex-specific Association of Interferon Regulatory Factor 4 with Childhood Acute Lymphoblastic Leukemia (ALL). 35th Annual American Society for Histocompatibility and Immunogenetics (ASHI) Meeting.
2. **Ucisik-Akkaya E, Davis C, Do T, and Dorak MT.** November 2-6, 2009. Immunoregulatory Gene Polymorphisms and Childhood Acute Lymphoblastic Leukemia (ALL) Susceptibility. 35th Annual American Society for Histocompatibility and Immunogenetics (ASHI) Meeting.
3. **Davis C, Ucisik-Akkaya E, Do T, and Dorak MT.** November 2-6, 2009. Polymorphisms of Iron Regulatory Genes with Immune Functions are Associated with Childhood Acute Lymphoblastic Leukemia (ALL) Susceptibility. 35th Annual American Society for Histocompatibility and Immunogenetics (ASHI) Meeting..

MDL: Research & Development

1. **Hilbert D, Paulish T, Mordechai E, Adelson ME, Gygax SE, and Trama J.** 2009. Antimicrobial non-susceptibility of cervico-vaginal and rectal *Eschericia coli* isolates is associated with phylogeny and plasmid carriage. *European Journal of Clinical Microbiology and Infectious Diseases.* **28(11):** 1399-403.
2. **Pena, K., Adelson, M.E. Mordechai, E, and Blaho, J.** 2009. Rapid isolation of HSV-1 and HSV-2 from OneSwab cervicovaginal specimens. *Journal of Virol. Methods*. **159(2):** 146-151.
3. **Ingvarsdottir, K and Blaho, J.** 2009. The role of chromatin in the regulation of HSV-1 viral gene expression and replication. *Future Microbiology*, **4:** 703-12.
4. **Ingvarsdottir K and Blaho, J.** 2009. Association of the herpes simplex virus major tegument structural protein VP22 with chromatin. *Biochim Biophys Acta*. Aug 12. *Epub ahead of print.*

Quality Assurance Q&A

Q: Does MDL send positive H1N1 specimens to State Department of Health Laboratories?

A: Currently no states are accepting H1N1 specimens; however, depending on the state requirements MDL is reporting positive results to State Departments of Health. Please contact Quality Control for more information.

If you have a question you would like addressed in future issues, please email your question(s) to QAQ&A@mdlab.com

e-Quiz

For results to the electronic Epidemiology Quiz, please visit www.mdlab.com and click on the e-Quiz link.

1. **True or False.** The currently circulating H1N1 strain of influenza is a major health concern because there is little to no existing immunity against this newly emergent viral strain.
2. **True or False.** Pregnant women are not at greater risk of complication as a result of infection with the 2009 H1N1 virus.
3. **True or False.** Vaccination and improved personal hygiene are the best defenses in combating H1N1 infection.
4. **True or False.** The 2009 H1N1 virus emerged as a result of recombination events among multiple influenza A strains.
5. **True or False.** Prophylactic use of the antivirals, oseltamivir and zanamivir, is recommended to limit the spread of H1N1 infection.

Medical Diagnostic Laboratories, L.L.C.

Test Announcement

Test 1125: 2009 H1N1 influenza virus (Swine Flu) with tamiflu resistance by Pyrosequencing.