

13 April 2010

*This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.*

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## **Chem-Bio News - Pandemic Influenza Edition #107**

- 1. DEVELOPMENT OF A REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR DETECTION OF PANDEMIC (H1N1) 2009 VIRUS AS A NOVEL MOLECULAR METHOD FOR DIAGNOSIS OF PANDEMIC INFLUENZA IN RESOURCE-LIMITED SETTINGS:** *"Use of the (H1N1) 2009 virus HA-specific RT-LAMP assay will enable the faster and easier diagnosis of pandemic (H1N1) 2009 virus infection, especially in resource-limited situations in developing countries."*
- 2. INFLUENZA VIRUS INACTIVATION FOR STUDIES OF ANTIGENICITY AND PHENOTYPIC NEURAMINIDASE INHIBITOR RESISTANCE PROFILING:** *"We demonstrated successful influenza virus characterization using formalin-and Triton X-100-inactivated virus samples. Application of these inactivation protocols limits work under BSL-3 conditions to virus culture, thus enabling more timely determination of public health impact and development of protective measures when a new influenza virus, e.g., pandemic A(H1N1)v virus, is introduced in humans."*
- 3. EFFECTS OF EARLY OSELTAMIVIR THERAPY ON VIRAL SHEDDING IN 2009 PANDEMIC INFLUENZA A (H1N1) VIRUS INFECTION:** *"When prescribed during the first 3 days of illness, oseltamivir shortened the duration of viral shedding."*
- 4. NOSOCOMIAL SWINE INFLUENZA (H1N1) PNEUMONIA: LESSONS LEARNED FROM AN ILLUSTRATIVE CASE:** *"This is the first known case of nosocomial paediatric transmission of H1N1 pneumonia."*
- 5. THE PANDEMIC 2009 (H1N1) SWINE INFLUENZA VIRUS IS MILD COMPARED TO THE PANDEMIC 1918 (H1N1) VIRUS BECAUSE OF A PROLINE-TO-SERINE SUBSTITUTION IN THE RECEPTOR-BINDING SITE OF ITS HEMAGGLUTININ - A HYPOTHESIS:** *"It is proposed that this substitution is the cause of the relative avirulence of the 2009 (H1N1) virus compared to the 1918 (H1N1) virus."*
- 6. ENTERIC ABSORPTION AND PHARMACOKINETICS OF OSELTAMIVIR IN CRITICALLY ILL PATIENTS WITH PANDEMIC (H1N1) INFLUENZA:** *"Adjustment of the dosage in patients with renal dysfunction requiring continuous renal replacement therapy is appropriate; adjustment for obesity does not appear to be necessary."*
- 7. SEVERITY OF PNEUMONIA DUE TO NEW H1N1 INFLUENZA VIRUS IN FERRETS IS INTERMEDIATE BETWEEN THAT DUE TO SEASONAL H1N1 VIRUS AND HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 VIRUS:** *"The new H1N1 virus replicated well throughout the lower respiratory tract and more extensively than did both seasonal H1N1 virus (which replicated mainly in the bronchi) and HPAI H5N1 virus (which replicated mainly in the alveoli)."*
- 8. DIET-INDUCED OBESITY IMPAIRS THE T CELL MEMORY RESPONSE TO INFLUENZA VIRUS INFECTION:** *"Furthermore, mRNA expression for IFN-gamma was >60% less in lungs of obese mice, along with one third the number of influenza-specific CD8(+) T cells producing IFN-gamma postsecondary infection versus lean controls. Memory CD8(+) T cells from obese mice had a >50% reduction in IFN-gamma production when stimulated with influenza-pulsed dendritic cells from lean mice."*
- 9. [CA] NEW EXPIRY DATE FOR UNUSED ADJUVANTED H1N1 VACCINE (AREPANRIX):** *"Based on the best scientific evidence available at the time of authorization in October 2009, a provisional expiry date of 18 months was set for Arepanrix. As a condition of the vaccine authorization, subsequent potency testing carried out by Health Canada and GlaxoSmithKline has determined that the expiry date for unused lots of Arepanrix should be revised to six months."*

**10. WHO ADMITS SHORTCOMINGS IN HANDLING FLU PANDEMIC:** *"The World Health Organization conceded shortcomings on Monday in its handling of the H1N1 swine flu pandemic, including a failure to communicate uncertainties about the new virus as it swept around the globe."*

## CB Daily Report

### Chem-Bio News

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#### **DEVELOPMENT OF A REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR DETECTION OF PANDEMIC (H1N1) 2009 VIRUS AS A NOVEL MOLECULAR METHOD FOR DIAGNOSIS OF PANDEMIC INFLUENZA IN RESOURCE-LIMITED SETTINGS**

Drug Week  
April 2, 2010

"This paper reports on the development of a one-step, real-time reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay targeting the hemagglutinin (HA) gene for the rapid molecular-based detection of pandemic (H1N1) 2009 virus. The detection limit of the pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay was same as that of the currently used real-time reverse transcription-PCR method."

"The assay detected the pandemic (H1N1) 2009 virus HA gene in 136 RNA samples extracted from nasopharyngeal swab specimens from Japanese and Vietnamese patients. No cross-reactive amplification with the RNA of other seasonal influenza viruses was observed, and the detection of specific viral genome targets in clinical specimens was achieved in less than 40 min. The sensitivity and specificity of the pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay obtained in this study were 97.8% and 100%, respectively."

"Use of the (H1N1) 2009 virus HA-specific RT-LAMP assay will enable the faster and easier diagnosis of pandemic (H1N1) 2009 virus infection, especially in resource-limited situations in developing countries."

The full article can be found at: (T. Kubo, et. al., "Development of a reverse transcription-loop-mediated isothermal amplification assay for detection of pandemic (H1N1) 2009 virus as a novel molecular method for diagnosis of pandemic influenza in resource-limited settings". Journal of Clinical Microbiology, 2010;48(3):728-35). Link not available.

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#### **INFLUENZA VIRUS INACTIVATION FOR STUDIES OF ANTIGENICITY AND PHENOTYPIC NEURAMINIDASE INHIBITOR RESISTANCE PROFILING**

FDA Law Weekly  
April 1, 2010

"We tested heat, formalin, Triton X-100, and beta-propiolactone treatments for their potencies in inactivating human influenza A(H3N2) and avian A(H7N3) viruses, as well as seasonal and pandemic A(H1N1) virus isolates, while allowing the specimens to retain their virological and immunological properties. Successful heat inactivation coincided with the loss of hemagglutinin (HA) and neuraminidase (NA) characteristics, and beta-propiolactone inactivation reduced the hemagglutination titer and NA activity of the human influenza virus 10-fold or more. Although Triton X-100 treatment resulted in inconsistent HA activity, the NA activities in culture supernatants were enhanced consistently. Nonetheless, formalin treatment permitted the best retention of HA and NA properties. Triton X-100 treatment proved to be the easiest-to-use influenza virus inactivation protocol for application in combination with phenotypic NA inhibitor susceptibility assays, while formalin treatment preserved B-cell and T-cell epitope antigenicity, allowing the detection of both humoral and cellular immune responses."

"We demonstrated successful influenza virus characterization using formalin-and Triton X-100-inactivated virus samples. Application of these inactivation protocols limits work under BSL-3 conditions to virus culture, thus enabling more timely determination of public health impact and development of protective measures when a new influenza virus, e.g., pandemic A(H1N1)v virus, is introduced in humans."

The full article can be found at: (M. Jonges, et. al., "Influenza virus inactivation for studies of antigenicity and phenotypic neuraminidase inhibitor resistance profiling". Journal of Clinical Microbiology, 2010;48(3):928-40). Link not available.

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## **EFFECTS OF EARLY OSELTAMIVIR THERAPY ON VIRAL SHEDDING IN 2009 PANDEMIC INFLUENZA A (H1N1) VIRUS INFECTION**

Medical Letter on the CDC & FDA

April 11, 2010

"During the pandemic containment response in Singapore, all patients with positive polymerase chain reaction (PCR) results for pandemic influenza (H1N1) 2009 were hospitalized, given oseltamivir for 5 days, and discharged when daily PCR results for combined nasal and throat swab samples became negative. Six patients had concurrent positive viral culture and PCR results. The median age of the first 70 consecutive patients was 26 years (interquartile range, 21-38 years); 60% were men, and 29% had comorbidity. The mean time ( $\pm$ SD) from illness onset to hospital admission was  $3\pm 2$  days. Influenza-like illness was noted in 63% of patients. Fever occurred in 91%, cough in 88%, sore throat in 66%, and rhinorrhea in 53% of patients. The mean duration ( $\pm$ SD) of viral shedding from illness onset was  $6\pm 2$  days. Viral shedding persisted beyond 7 days in 37% of patients. Clinical features and viral shedding were similar between those with and without comorbidity, except the former had more cough and lower oxygen saturation. Patients receiving oseltamivir on days 1 to 3 of illness had significantly shorter viral shedding duration, compared with those treated from day 4 onwards ( $p < .05$ ). The mean durations ( $\pm$ SD) of positive PCR and viral culture results were  $5\pm 8$  and  $4\pm 18$  days, respectively, for 6 patients with concurrent positive viral culture and PCR results. Prolonged viral shedding was noted in young immunocompetent adults with mild pandemic influenza (H1N1) 2009 despite receipt of oseltamivir."

"When prescribed during the first 3 days of illness, oseltamivir shortened the duration of viral shedding."

The full article can be found at: (L.M. Ling, et. al., "Effects of early oseltamivir therapy on viral shedding in 2009 pandemic influenza A (H1N1) virus infection". Clinical Infectious Diseases, 2010;50(7):963-9). Link not available.

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## **NOSOCOMIAL SWINE INFLUENZA (H1N1) PNEUMONIA: LESSONS LEARNED FROM AN ILLUSTRATIVE CASE**

World Disease Weekly

April 6, 2010

"In the spring of 2009, our institution found itself at the epicentre of the 'herald wave' of the swine influenza (H1N1) pandemic in New York. We were inundated with hundreds of patients exhibiting influenza-like illnesses (ILIs), presenting for rapid influenza A testing."

"During this pandemic, an infant with newly diagnosed acute lymphatic leukaemia (ALL) was admitted for induction chemotherapy. After being in hospital for a week, she developed high fever and shortness of breath, although her chest X-ray was clear. She was admitted to the paediatric intensive care unit (PICU) for mechanical ventilation. As we were in the midst of the pandemic, diagnosis of H1N1 pneumonia was considered and reverse transcription-polymerase chain reaction for H1N1 was positive. Contact investigation revealed that none of her family members/visitors had been in recent/close contact with anyone with ILI/H1N1. The investigation also revealed that paediatric healthcare staff, in

contact with H1N1 patients, had rotated into PICU to care for the patient. Although no specific individual could be identified, it seems likely that H1N1 was transmitted to the patient by a healthcare worker who worked both in the paediatric ward and the PICU."

"This is the first known case of nosocomial paediatric transmission of H1N1 pneumonia."

The full article can be found at: (B.A. Cunha, et. al., "Nosocomial swine influenza (H1N1) pneumonia: lessons learned from an illustrative case". Journal of Hospital Infection, 2010;74(3):278-81). Link not available.

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## **THE PANDEMIC 2009 (H1N1) SWINE INFLUENZA VIRUS IS MILD COMPARED TO THE PANDEMIC 1918 (H1N1) VIRUS BECAUSE OF A PROLINE-TO-SERINE SUBSTITUTION IN THE RECEPTOR-BINDING SITE OF ITS HEMAGGLUTININ - A HYPOTHESIS**

Biotech Week

March 31, 2010

"The relative mildness of the pandemic 2009 (H1N1) swine influenza virus compared to the 1918 pandemic (H1N1) virus may be due to a variety of possible causes, including the existence of effective immunity in the host, the lessened ability of the virus to bind to target cells or to replicate in them, a diminished secretion of molecules that could cause further complications like pneumonia, etc. A comparison of the hemagglutinin sequences from the pandemic 2009 (H1N1) viruses with that of the 1918 (H1N1) virus reveals a difference in the residues occupying position 200, which has been shown to be involved in receptor binding."

"In all the pandemic 2009 (H1N1) hemagglutinin sequences available in the NCBI database, position 200 is occupied by serine. In the hemagglutinin of the 1918 (H1N1) virus, position 200 is occupied by proline. A proline-to-serine substitution could introduce a significant structural change in the receptor-binding site of the hemagglutinin, which could reduce the receptor-binding ability of the 2009 (H1N1)virus."

"It is proposed that this substitution is the cause of the relative avirulence of the 2009 (H1N1) virus compared to the 1918 (H1N1) virus."

The full article can be found at: (E.A. Padlan, et. al., "The pandemic 2009 (H1N1) swine influenza virus is mild compared to the pandemic 1918 (H1N1) virus because of a proline-to-serine substitution in the receptor-binding site of its hemagglutinin - A hypothesis". Medical Hypotheses, 2010;74(2):240-241). Link not available.

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## **ENTERIC ABSORPTION AND PHARMACOKINETICS OF OSELTAMIVIR IN CRITICALLY ILL PATIENTS WITH PANDEMIC (H1N1) INFLUENZA**

Virus Weekly

April 6, 2010

"We included 41 patients 18 years of age and older with suspected or confirmed pandemic (H1N1) influenza who were admitted for ventilatory support to nine ICUs in three cities in Canada and Spain. Using tandem mass spectrometry, we assessed plasma levels of oseltamivir free base and its active metabolite carboxylate at baseline (before gastric administration of the drug) and at 2, 4, 6, 9 and 12 hours after the fourth or later dose. Among the 36 patients who did not require dialysis, the median concentration of oseltamivir free base was 10.4 (interquartile range [IQR] 4.8-14.9)  $\mu\text{g/L}$ ; the median concentration of the carboxylate metabolite was 404 (IQR 257-900)  $\mu\text{g/L}$ . The volume of distribution of the carboxylate metabolite did not increase with increasing body weight ( $R^2 = 0.00$ ,  $p = 0.87$ ). The rate of elimination of oseltamivir carboxylate was modestly correlated with estimations of creatinine clearance ( $R^2 = 0.27$ ,  $p < 0.001$ ). Drug clearance in the five patients who required continuous renal replacement therapy was about one-sixth that in the 36 patients with relatively normal renal function. Oseltamivir was well absorbed enterically in critically ill patients admitted to the ICU with suspected or

confirmed pandemic (H1N1) influenza. The dosage of 75 mg twice daily achieved plasma levels that were comparable to those in ambulatory patients and were far in excess of concentrations required to maximally inhibit neuraminidase activity of the virus.”

“Adjustment of the dosage in patients with renal dysfunction requiring continuous renal replacement therapy is appropriate; adjustment for obesity does not appear to be necessary.”

The full article can be found at: (R.E. Ariano, et. al., “Enteric absorption and pharmacokinetics of oseltamivir in critically ill patients with pandemic (H1N1) influenza”. Canadian Medical Association Journal, 2010;182(4):357-363). Link not available.

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### **SEVERITY OF PNEUMONIA DUE TO NEW H1N1 INFLUENZA VIRUS IN FERRETS IS INTERMEDIATE BETWEEN THAT DUE TO SEASONAL H1N1 VIRUS AND HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 VIRUS**

World Disease Weekly

April 6, 2010

“However, the ability of the new H1N1 virus to cause pneumonia is poorly understood. The new H1N1 virus was inoculated intratracheally into ferrets. Its ability to cause pneumonia was compared with that of seasonal influenza H1N1 virus and highly pathogenic avian influenza (HPAI) H5N1 virus by using clinical, virological, and pathological analyses. Our results showed that the new H1N1 virus causes pneumonia in ferrets intermediate in severity between that caused by seasonal H1N1 virus and by HPAI H5N1 virus. The new H1N1 virus replicated well throughout the lower respiratory tract and more extensively than did both seasonal H1N1 virus (which replicated mainly in the bronchi) and HPAI H5N1 virus (which replicated mainly in the alveoli). High loads of new H1N1 virus in lung tissue were associated with diffuse alveolar damage and mortality.”

The full article can be found at: (J.M. van den Brand, et. al., “Severity of pneumonia due to new H1N1 influenza virus in ferrets is intermediate between that due to seasonal H1N1 virus and highly pathogenic avian influenza H5N1 virus”. Journal of Infectious Diseases, 2010;201(7):993-9). Link not available.

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### **DIET-INDUCED OBESITY IMPAIRS THE T CELL MEMORY RESPONSE TO INFLUENZA VIRUS INFECTION**

World Disease Weekly

April 6, 2010

“In male, diet-induced obese C57BL/6 mice, a secondary H1N1 influenza challenge following a primary H3N2 infection led to a 25% mortality rate (with no loss of lean controls), 25% increase in lung pathology, failure to regain weight, and 10-to 100-fold higher lung viral titers. Furthermore, mRNA expression for IFN-gamma was >60% less in lungs of obese mice, along with one third the number of influenza-specific CD8(+) T cells producing IFN-gamma postsecondary infection versus lean controls. Memory CD8(+) T cells from obese mice had a >50% reduction in IFN-gamma production when stimulated with influenza-pulsed dendritic cells from lean mice. Thus, the function of influenza-specific memory T cells is significantly reduced and ineffective in lungs of obese mice.”

The full article can be found at: (E.A. Karlsson, et. al., “Diet-induced obesity impairs the T cell memory response to influenza virus infection”. Journal of Immunology, 2010;184(6):3127-33). Link not available.

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### **[CA] NEW EXPIRY DATE FOR UNUSED ADJUVANTED H1N1 VACCINE (AREPANRIX**

Health Canada

April 09, 2010

“Health Canada, in consultation with GlaxoSmithKline, has revised the provisional expiry date of the adjuvanted H1N1 vaccine (Arepanrix). This revision is not safety-related but is due to a decline in strength of the H1N1 antigen in specific lots.

Based on the best scientific evidence available at the time of authorization in October 2009, a provisional expiry date of 18 months was set for Arepanrix. As a condition of the vaccine authorization, subsequent potency testing carried out by Health Canada and GlaxoSmithKline has determined that the expiry date for unused lots of Arepanrix should be revised to six months.”

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“Health Canada will re-initiate release of Arepanrix lots for domestic or international use that show the revised expiry date.

This revision does not affect the GlaxoSmithKline's unadjuvanted H1N1 vaccine "Influenza A (H1N1) 2009 Pandemic Monovalent Vaccine (Without Adjuvant)." The expiry date for Canada's unadjuvanted H1N1 vaccine remains at 18 months. Health Canada and GlaxoSmithKline are continuing to monitor the situation.”

The full article can be found at: [http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/\\_2010/2010\\_54-eng.php](http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/_2010/2010_54-eng.php)

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## **WHO ADMITS SHORTCOMINGS IN HANDLING FLU PANDEMIC**

By Stephanie Nebehay

Reuters

April 12, 2010

“The World Health Organization conceded shortcomings on Monday in its handling of the H1N1 swine flu pandemic, including a failure to communicate uncertainties about the new virus as it swept around the globe.

Keiji Fukuda, the WHO's top influenza expert, said the U.N. agency's six-phase system for declaring a pandemic had sown confusion about the flu bug which was ultimately not as deadly as the widely-feared avian influenza.”

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“Critics have said the WHO created panic about the swine flu virus, which turned out to be moderate in its effect, and caused governments to stockpile vaccines which went unused.

Some questioned its links to the pharmaceutical industry after companies like GlaxoSmithKline and Sanofi-Aventis profited from producing H1N1 vaccine.”

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“The World Bank has estimated that countries have spent \$4 billion to prepare pandemic preparedness plans and respond to the outbreaks, according to the U.S. delegation to the talks.”

The full article can be found at: <http://www.reuters.com/article/idUSTRE63B2TL20100412>

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