22 June 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 #117

1. ASSESSMENT OF THE REMOVAL AND INACTIVATION OF INFLUENZA VIRUSES H5N1 AND H1N1 BY DRINKING WATER TREATMENT: *"Of the chemical disinfection treatments, ozone, chlorine and chlorine dioxide were all very effective in inactivating H5N1 and H1N1, whereas monochloramine treatment required higher doses and longer contact times to achieve significant reductions."*

2. HIGH-LEVEL EXPRESSION, PURIFICATION AND CHARACTERIZATION OF CODON-OPTIMIZED RECOMBINANT HEMAGGLUTININ 5 PROTEINS IN MAMMALIAN CELLS: *"Purification of H5 proteins from mammalian cells could be used for large-scale production of recombinant H5 protein for basic scientific research or the development of vaccines."*

3. ENHANCEMENT OF REVERSE GENETICS-DERIVED SWINE-ORIGIN H1N1 INFLUENZA VIRUS SEED VACCINE GROWTH BY INCLUSION OF INDIGENOUS POLYMERASE PB1 PROTEIN: *"Furthermore, it was found that growth enhancement by PB1 was specific for reassortant harboring HA of S-OIV, suggesting that the slow growth property of S-OIV reassortant virus is possibly due to restrictions imposed by the HA gene."*

4. COMPARISON OF THE PERFORMANCE OF DIRECT FLUORESCENT ANTIBODY STAINING, A POINT-OF-CARE RAPID ANTIGEN TEST AND VIRUS ISOLATION WITH THAT OF RT-PCR FOR THE DETECTION OF NOVEL 2009 INFLUENZA A (H1N1) VIRUS IN RESPIRATORY SPECIMENS: *"In summary, DFA staining is superior to POC testing and may be appropriate for patients expected to have a rather high level of virus replication."*

5. A SIMPLE METHOD FOR MOLECULAR DETECTION OF SWINE-ORIGIN AND HUMAN-ORIGIN INFLUENZA A VIRUS. VECTOR - BORNE AND ZOONOTIC DISEASES: *"Finally, in silico analysis of databases predicted that minor modifications of the van Elden primers would facilitate the use of this assay for the broad spectrum detection of all currently characterized variants of influenza A virus, including avian strains."*

6. INFLUENZA-INDUCED PRODUCTION OF INTERFERON-ALPHA IS DEFECTIVE IN GERIATRIC INDIVIDUALS: *"Influenza-induced production of IFN-alpha was defective in geriatric PBMCs by a mechanism that was independent of reduced pDC frequency or viability, defects in uptake of influenza, inability to secrete IFN-alpha, or defects in TLR7 signaling."*

7. COMPUTER-AIDED INFLUENZA VIRUS VACCINE CREATED: *"A team of molecular biologists and computer scientists at Stony Brook University have used a novel method to weaken (attenuate) influenza virus by way of designing hundreds of mutations to its genetic code to create an effective vaccine."*

8. NEW STUDIES SHOW LOW HOUSEHOLD TRANSMISSION OF H1N1: *"Two new reports in the medical literature today suggest that secondary transmission of the pandemic H1N1 virus in households is low, which seems to confirm earlier reports that the virus is less contagious than past pandemic viruses and some seasonal flu viruses."*

CB Daily Report

Chem-Bio News

ASSESSMENT OF THE REMOVAL AND INACTIVATION OF INFLUENZA VIRUSES H5N1 AND H1N1 BY DRINKING WATER TREATMENT
Energy & Ecology
June 18, 2010

"As those viruses could possibly contaminate water resources through wild birds excreta or through sewage, the aim of our work was to find out whether the treatment processes in use in the drinking water industry are suitable for eradicating them. The effectiveness of physical treatments (coagulation-flocculation-settling, membrane ultrafiltration and ultraviolet) was assessed on H5N1, and that of disinfectants (monochloramine, chlorine dioxide, chlorine, and ozone) was established for both the H5N1 and H1N1 viruses. Natural water samples were spiked with human H5N1/H1N1 viruses. For the coagulation-settling experiments, raw surface water was treated in jar-test pilots with 3 different coagulating agents (aluminum sulfate, ferric chloride, aluminum polychorosulfate). Membrane performance was quantified using a hollow-fiber ultrafiltration system. Ultraviolet irradiation experiments were conducted with a collimated beam that made it possible to assess the effectiveness of various UV doses (25-60 mJ/cm(2)). In the case of ozone, 0.5 mg/L and 1 mg/L residual concentrations were tested with a contact time of 10 min. Finally, for chlorine, chlorine dioxide and monochloramine treatments, several residual oxidant target levels were tested (from 0.3 to 3 mg/L) with contact times of 5-120 min. The infectivity of the H5N1 and H1N1 viruses in water samples was quantified in cell culture using a microtiter endpoint titration. The impact of coagulation-settling on the H5N1 subtype was quite low and variable. In contrast, ultrafiltration achieved more than a 3-log reduction (and more than a 4-log removal in most cases), and UV treatment was readily effective on its inactivation (more than a 5-log inactivation with a UV dose of 25 mJ/cm(2)). Of the chemical disinfection treatments, ozone, chlorine and chlorine dioxide were all very effective in inactivating H5N1 and H1N1, whereas monochloramine treatment required higher doses and longer contact times to achieve significant reductions. Our findings suggest that the water treatment strategies that are currently used for surface water treatment are entirely suitable for removing and/or inactivating influenza A viruses."

The full article can be found at: (D. Lenes, et. al., "Assessment of the removal and inactivation of influenza viruses H5N1 and H1N1 by drinking water treatment". Water Research, 2010;44(8):2473-2486). Link not available.
Return to Top

HIGH-LEVEL EXPRESSION, PURIFICATION AND CHARACTERIZATION OF CODON-OPTIMIZED RECOMBINANT HEMAGGLUTININ 5 PROTEINS IN MAMMALIAN CELLS
Drug Week
June 18, 2010

"Hemagglutinin is the membrane glycoprotein of avian influenza virus responsible for receptor binding to human cells and the main immunogenic protein that elicits a strong immune response. Although this protein is of great importance to the study of pathogenesis and vaccine development, its expression and purification are difficult due to high levels of glycosylation. In this study, we expressed codon-optimized, full-length hemagglutinin 5 (H5) protein fused with a human IgG Fc tag (H5-Fc) in HEK293 cells. To enhance secretion of this protein, we also deleted the transmembrane domain and the intracellular domain of the H5 protein (H5DeltaTM-Fc). Purified proteins were obtained using a protein A column. ELISA revealed that the yield of soluble H5DeltaTM-Fc protein in the supernatant was about 20 mg/L. Western blotting and fluorescence activated cell sorter (FACS) indicated that the purified H5 protein was correctly folded and biologically active."

"Purification of H5 proteins from mammalian cells could be used for large-scale production of recombinant H5 protein for basic scientific research or the development of vaccines."

The full article can be found at: (J.L. Yang, et. al., "High-level expression, purification and characterization of codon-optimized recombinant hemagglutinin 5 proteins in mammalian cells". Chinese Medical Journal, 2010;123(8):1073-7). Link not available.
Return to Top

ENHANCEMENT OF REVERSE GENETICS-DERIVED SWINE-ORIGIN H1N1 INFLUENZA VIRUS SEED VACCINE GROWTH BY INCLUSION OF INDIGENOUS POLYMERASE PB1 PROTEIN
Obesity, Fitness & Wellness Week
June 12, 2010

"However, reassortant viruses containing 6 internal genes from A/PR/8/34 and the hemagglutinin (HA) and neuraminidase (NA) genes from S-OIV showed very slow growth characteristics, hampering the speed of vaccine production. Here, we showed that the reverse genetics-derived H1N1 could be rescued with sensible viral titer by replacing PB1 of A/PR/8/34 with that of S-OIV for plasmid transfection. The ''5+3'' reassortant viruses have shown higher growth rate after transfection compared to that of ''6+2'' reassortant. The difference between PB1 of S-OIV and that of A/PR/8/34 in terms of the enhancement of virus growth was possibly due to the augmentation of viral polymerase activity, but not the lack of functional FB1-F2."

"Furthermore, it was found that growth enhancement by PB1 was specific for reassortant harboring HA of S-OIV, suggesting that the slow growth property of S-OIV reassortant virus is possibly due to restrictions imposed by the HA gene."

The full article can be found at: (A. Wanitchang, et. al., "Enhancement of reverse genetics-derived swine-origin H1N1 influenza virus seed vaccine growth by inclusion of indigenous polymerase PB1 protein". Virus Research, 2010;147(1):145-148). Link not available.
Return to Top

COMPARISON OF THE PERFORMANCE OF DIRECT FLUORESCENT ANTIBODY STAINING, A POINT-OF-CARE RAPID ANTIGEN TEST AND VIRUS ISOLATION WITH THAT OF RT-PCR FOR THE DETECTION OF NOVEL 2009 INFLUENZA A (H1N1) VIRUS IN RESPIRATORY SPECIMENS
Ecology, Environment & Conservation
June 11, 2010

"In contrast to seasonal influenza, where point-of-care (POC) rapid antigen tests and direct fluorescent antibody (DFA) staining ensure rapid detection, diagnosis of A/H1N1/2009 has so far been based on RT-PCR. This study retrospectively compared the performance of the Quidel QuickVue POC test, DFA staining and virus isolation with that of RT-PCR for A/H1N1/2009 detection in 526 respiratory specimens collected during the first wave of the outbreak from May to September 2009. A/H1N1/2009 was detected in 9.1% (48/526) of samples. One hundred and thirty-seven of the A/H1N1/2009 PCR-negative samples were additionally tested using a RealAccurate Respiratory RT-PCR panel, revealing other respiratory viruses (mainly entero/rhino-and adenoviruses) in 42.3% (58/137). All methods analysed detected A/H1N1/2009 with excellent specificity but different sensitivities (POC test: 18.2%; DFA staining: 38.7%; virus isolation: 45.7%). Therefore, the POC test was not suitable for diagnosis, detecting A/H1N1/2009 only if present in high concentrations (corresponding median Ct value=19.0; range=16.5-21.4). DFA staining was also able to detect A/H1N1/2009 in specimens with a lower virus concentration (median Ct value=24.0; range=16.5-29.8). Virus isolation, which was positive after a median time of 7.5 days, was too time-consuming. In summary, DFA staining is superior to POC testing and may be appropriate for patients expected to have a rather high level of virus replication."

The full article can be found at: (T. Ganzenmueller, et. al., "Comparison of the performance of direct fluorescent antibody staining, a point-of-care rapid antigen test and virus isolation with that of RT-PCR for the detection of novel 2009 influenza A (H1N1) virus in respiratory specimens". Journal of Medical Microbiology, 2010;59(Pt 6):713-7). Link not available.
Return to Top

A SIMPLE METHOD FOR MOLECULAR DETECTION OF SWINE-ORIGIN AND HUMAN-ORIGIN INFLUENZA A VIRUS. VECTOR - BORNE AND ZOONOTIC DISEASES
Science Letter
June 15, 2010

"Here we present a real-time one-step reverse transcriptase-polymerase chain reaction SYBR Green assay derived from the method reported by van Elden and collaborators (2001) that ensures the rapid, sensitive, and cost-effective detection of both seasonal influenza A virus and emerging (H1N1) swine-origin influenza A virus (S-OIV)."

"In addition to this screening test, which successfully detected both seasonal influenza A virus and S-OIV in human clinical samples, we showed that the probe initially designed by van Elden and collaborators could detect seasonal influenza A virus, but not S-OIV; a new probe was designed and tested that specifically detects S-OIV, but not seasonal influenza A. Both probe-based assays were validated by testing human clinical samples and specifically detected either seasonal influenza virus or S-OIV."

"Finally, in silico analysis of databases predicted that minor modifications of the van Elden primers would facilitate the use of this assay for the broad spectrum detection of all currently characterized variants of influenza A virus, including avian strains."

The full article can be found at: (L. Ninove, et. al., "A Simple Method for Molecular Detection of Swine-Origin and Human-Origin Influenza A Virus. Vector - Borne and Zoonotic Diseases", 2010;10(3):237-240). Link not available.
Return to Top

INFLUENZA-INDUCED PRODUCTION OF INTERFERON-ALPHA IS DEFECTIVE IN GERIATRIC INDIVIDUALS
Science Letter
June 15, 2010

"Our aim was to analyze interferon-alpha (IFN-alpha) production in peripheral blood mononuclear cells (PBMCs) isolated from young and geriatric adult donors, stimulated with influenza A or Toll-like receptor (TLR) ligands. IFN-alpha is a signature anti-viral cytokine that also shapes humoral and cell-mediated immune responses. Geriatric PBMCs produced significantly less IFN-alpha in response to live or inactivated influenza (a TLR7 ligand) but responded normally to CpG ODN (TLR9 ligand) and Guardiquimod (TLR7 ligand). All three ligands activate plasmacytoid dendritic cells (pDCs). While there was a modest decline in pDC frequency in older individuals, there was no defect in uptake of influenza by geriatric pDCs."

"Influenza-induced production of IFN-alpha was defective in geriatric PBMCs by a mechanism that was independent of reduced pDC frequency or viability, defects in uptake of influenza, inability to secrete IFN-alpha, or defects in TLR7 signaling."

The full article can be found at: (D.H. Canaday, et. al., "Influenza-induced production of interferon-alpha is defective in geriatric individuals". Journal of Clinical Immunology, 2010;30(3):373-83). Link not available.
Return to Top

COMPUTER-AIDED INFLUENZA VIRUS VACCINE CREATED
Infection Control Today Magazine
June 16, 2010

"A team of molecular biologists and computer scientists at Stony Brook University have used a novel method to weaken (attenuate) influenza virus by way of designing hundreds of mutations to its genetic code to create an effective vaccine.

Reported online and in the July issue of Nature Biotechnology, the method may be a major step in

developing more effective and safe vaccines against influenza, which claims 250,000 to 500,000 lives annually worldwide, partly because existing vaccines are not fully effective.

The research is an outgrowth of years of investigation by a team headed by Eckard Wimmer, PhD, Distinguished Professor, Department of Molecular Genetics and Microbiology at Stony Brook University. In 2002, Wimmer and colleagues synthesized and generated poliovirus, the first artificial synthesis of any virus. Two years ago, they designed and synthesized a new class of attenuated polio viruses. Viruses attenuated by traditional means often make effective vaccines but sometimes mutate to regain virulence. The creation of synthetic viruses nearly eliminates the possibility of the virus regaining virulence.

In their latest research, the same method that the team used to create weakened synthetic polio viruses was employed to design an influenza vaccine. They found this vaccine effective and safe against influenza in mice."

The full article can be found at: http://www.infectioncontroltoday.com/hotnews/computer-aided-influenza-virus.html
Return to Top

NEW STUDIES SHOW LOW HOUSEHOLD TRANSMISSION OF H1N1
By Lisa Schnirring
CIDRAP News (Center for Infectious Disease Research & Policy – University of Minnesota)
June 21, 2010

"Two new reports in the medical literature today suggest that secondary transmission of the pandemic H1N1 virus in households is low, which seems to confirm earlier reports that the virus is less contagious than past pandemic viruses and some seasonal flu viruses.

One study looked at household transmission rates in boys who got sick with pandemic flu at a residential camp in Florida last summer, and another explored rates in the households of students sickened in a Hong Kong secondary school outbreak in June 2009. Both reports appear today in the early online edition of Epidemiology and Infection.

In the first study, 49 of 212 boys at the summer camp had confirmed or probable pandemic H1N1 infections, putting the primary attack rate at 23%. Among 87 household contacts, the investigators observed three instances of probable secondary transmission, for an attack rate of 3.5%. They reported that all three secondary cases occurred in households where the sick camper returned home 1 day after illness onset.

The authors suggested that two factors contributed to the low secondary attack rate they observed: returning home after peak infectivity and advance warning before the sick campers' return.

In the second study, Hong Kong researchers found that 65 of 511 students got sick in the outbreak at the secondary school. They found 12 confirmed H1N1 infections among 205 of the sick students' household contacts for a secondary attack rate of 5.9%. The group found that household contacts younger than 18 were 15 times more likely to be infected than older contacts.

Researchers also explored the role of oseltamivir (Tamiflu) in protecting household contacts. They found that contacts who had received oseltamivir prophylaxis were less likely to get sick."

The full article can be found at:
http://www.cidrap.umn.edu/cidrap/content/influenza/swineflu/news/jun2110household-br.html
Return to Top

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