

6 April 2010

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## **Chem-Bio News – Pandemic Influenza Edition #106**

### **1. LYMPHOCYTE TO MONOCYTE RATIO AS A SCREENING TOOL FOR INFLUENZA:**

*"A ratio of lymphocytes to monocytes below 2 is proposed as a screening tool for influenza infection instead of rapid tests."*

### **2. RAPID INFLUENZA ANTIGEN TEST FOR DIAGNOSIS OF PANDEMIC (H1N1)**

**2009:** *"We compared the QuickVue Influenza Test with PCR for diagnosing pandemic (H1N1) 2009 in 404 persons with influenza-like illness. Overall sensitivity, specificity, and positive and negative predictive values were 66%, 84%, 84%, and 64%, respectively. Rapid test results should be interpreted cautiously when pandemic (H1N1) 2009 virus is suspected."*

### **3. STRATEGIES FOR MITIGATING AN INFLUENZA PANDEMIC WITH PRE-**

**PANDEMIC H5N1 VACCINES:** *"We showed that by effectively moving the delay between first and second doses into the pre-pandemic period, the split vaccination strategy achieved a substantially better attack rate reduction than the reactive strategy."*

### **4. DUPLEX REAL-TIME REVERSE TRANSCRIPTASE PCR ASSAYS FOR RAPID DETECTION AND IDENTIFICATION OF PANDEMIC (H1N1) 2009 AND SEASONAL INFLUENZA A/H1, A/H3, AND B VIRUSES:**

*"Minimum sensitivities and specificities were 98.8% and 100%, respectively, for pandemic (H1N1) 2009, 81.5% and 98.9% for seasonal A/H1, and 96.3% and 99.6% for A/H3."*

### **5. AN ALTERNATIVE METHOD FOR PREPARATION OF PANDEMIC INFLUENZA STRAIN-SPECIFIC ANTIBODY FOR VACCINE POTENCY DETERMINATION:**

*"The results demonstrate a feasible approach for addressing one of the potential bottlenecks in inactivated pandemic influenza vaccine production and are particularly important in light of the difficulties in preparation of potency reagent antibody for pandemic H1N1 (2009) virus vaccines."*

### **6. DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC ASSAY SPECIFICALLY DETECTING PANDEMIC H1N1 (2009) INFLUENZA VIRUS:**

*"Preliminary evaluation of clinical samples from 5 individuals with PCR-confirmed human AH1pdm infection showed that the RDK was positive for all samples, with the same detection intensity as that of a*

# CB Daily Report

## Chem-Bio News

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### LYMPHOCYTE TO MONOCYTE RATIO AS A SCREENING TOOL FOR INFLUENZA

By George Merekoulias, Evangelos C Alexopoulos, Theodore Belezos, Eugenia

Panagiotopoulou et al.

PLoS Currents – Influenza

April 01, 2010

"In fall 2009 the emergency department of a clinic in Greece with increased patient visits due to influenza-like illness observed a particular pattern in the complete blood count (CBC) of these patients. In 90% of all patients with probable influenza, lymphopenia and/or monocytosis were present. Relative lymphopenia with or without monocytosis appears to be a laboratory marker for H1N1 virus infection, a finding that could play a major role in early identifying and treating patients with new influenza A. A ratio of lymphocytes to monocytes below 2 is proposed as a screening tool for influenza infection instead of rapid tests."

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"Given the fact that the hematology analyzer cannot discriminate between monocytes and large lymphocytes this study cannot suggest the presence of actual relative monocytosis, however the use of the relevant ratio in the emergency department is independent of that fact. A point score system for the probability of H1N1 infection, involving elements of CBC and clinical criteria has already been proposed for hospitalised patients [14]. At present, a decrease in the number of cases in Greece is observed, probably due to milder weather conditions. However, as lower temperatures in Greece are expected in March and April, a new pandemic wave could stress the health system again, considering the fact, that seasonal influenza shows usually a peak at the end of the winter months in Greece [13].

The latest update from WHO [15] points that up to February 2010, worldwide more than 212 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1, including more than 15000 deaths, (4000 in Europe). In Europe, although pandemic influenza virus continues to circulate widely, particularly across central, southern, and eastern Europe, the overall intensity of pandemic influenza activity has declined substantially from peaks of activity seen earlier during the winter transmission period. There are more active areas of transmission like Northern Africa, South Asia, and East Asia [15].

However, even if a new pandemic wave does not occur, one should be still aware of the new influenza virus, because of its tendency to affect the lower respiratory tract [16]. Thus, a simple diagnostic tool as the proposed, can prove to be valuable in a low activity period, mainly in patients with influenza-like symptoms.

Finally, a ratio of lymphocytes to monocytes below 2, is considered indicative of the 'turn' in

the parameters of CBC. However, the optimal cut-off point should be determined based on the RT-PCR as gold-standard diagnostic test.

We suggest this observation to be investigated in larger study populations including smaller age groups and performing RT-PCR and microscopic analysis of CBC in order to verify, if CBC and especially relative monocytosis could be applied as a time-saving and cost-effective screening test for H1N1 virus infection, leading to early antiviral treatment and hence to a decrease in the incidence of complicated cases. Such a tool would be very helpful in areas where laboratory confirmation is limited due to financial restrictions or excess demand."

The full article can be found at: <http://knol.google.com/k/george-merekoulias/lymphocyte-to-monocyte-ratio-as-a/wwim31ohhvdI/1?collectionId=28qm4w0q65e4w.1&position=1#>

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## **RAPID INFLUENZA ANTIGEN TEST FOR DIAGNOSIS OF PANDEMIC (H1N1) 2009**

By Janice K. Louie, Hugo Guevara, Erica Boston, Melissa Dahlke, Maria Nevarez, Tong Kong, Robert Schechter, Carol A. Glaser, and David P. Schnurr

Emerging Infectious Diseases

ePub ahead of print April 2010

"We compared the QuickVue Influenza Test with PCR for diagnosing pandemic (H1N1) 2009 in 404 persons with influenza-like illness. Overall sensitivity, specificity, and positive and negative predictive values were 66%, 84%, 84%, and 64%, respectively. Rapid test results should be interpreted cautiously when pandemic (H1N1) 2009 virus is suspected."

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"In conclusion, we found the QuickVue influenza test had suboptimal sensitivity and specificity for the detection of pandemic (H1N1) 2009 during a period of increased prevalence in California. This finding suggests that rapid test results that may lead to changes in clinical management or public health intervention should be confirmed with PCR. A strength of our study is its reflection of typical testing practices in outpatient settings and the need for reconsideration of the clinical application of rapid test results. The development of more accurate point-of-care tests for seasonal and pandemic (H1N1) 2009 infection is urgently needed."

The full article can be found at: <http://www.cdc.gov/eid/content/16/5/pdfs/09-1894.pdf>

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## **STRATEGIES FOR MITIGATING AN INFLUENZA PANDEMIC WITH PRE-PANDEMIC H5N1 VACCINES**

Food & Drug Law Weekly

April 2, 2010

"Given that recently developed candidate pre-pandemic H5N1 vaccines have shown potential for cross-strain protection, we investigated alternative vaccination strategies that exploit such vaccines using an agent-based simulation model of an actual community of approximately 30 000 people in a developed country. Assuming that a two-dose vaccination regimen would be required, we examined three vaccination strategies: pre-emptive, with vaccination applied prior to emergence of human-transmissible H5N1 influenza; reactive, where vaccination was initiated immediately after the first cases in the community were diagnosed; and a 'split' strategy where the first dose was administered pre-emptively during the pre-pandemic phase, with the second dose administered reactively. We showed that by effectively moving the delay between first and second doses into the pre-pandemic period, the split vaccination strategy achieved a substantially better attack rate reduction than the reactive strategy."

The full article can be found at: (G. Milne, et. al., "Strategies for mitigating an influenza pandemic with pre-pandemic H5N1 vaccines". *Journal of the Royal Society, Interface*, 2010; 7 (45):573-86). Link not available.

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## **DUPLEX REAL-TIME REVERSE TRANSCRIPTASE PCR ASSAYS FOR RAPID DETECTION AND IDENTIFICATION OF PANDEMIC (H1N1) 2009 AND SEASONAL INFLUENZA A/H1, A/H3, AND B VIRUSES**

Genetics & Environmental Business Week

April 1, 2010

"A real-time reverse transcriptase PCR (RT-PCR) assay for the detection of pandemic (H1N1) 2009 was designed and used with existing real-time RT-PCR assays for seasonal influenza viruses A and B. MS2 coliphage was added to all samples and amplified as a quality control. Three duplex RT-PCR assays, each containing two primer pairs and corresponding 5' nuclease probes, were initially evaluated on control material and stored samples and showed high sensitivity and specificity. More than 11,000 clinical samples were then tested for influenza A and B matrix gene targets and specific hemagglutinin gene targets for seasonal influenza A/H1, A/H3, and pandemic A (H1N1) 2009. Minimum sensitivities and specificities were 98.8% and 100%, respectively, for pandemic (H1N1) 2009, 81.5% and 98.9% for seasonal A/H1, and 96.3% and 99.6% for A/H3."

The full article can be found at: (G. Chidlow, et. al., "Duplex real-time reverse transcriptase PCR assays for rapid detection and identification of pandemic (H1N1) 2009 and seasonal influenza A/H1, A/H3, and B viruses". *Journal of Clinical Microbiology*, 2010; 48(3):862-6). Link not available.

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## **AN ALTERNATIVE METHOD FOR PREPARATION OF PANDEMIC INFLUENZA STRAIN-**

## **SPECIFIC ANTIBODY FOR VACCINE POTENCY DETERMINATION**

Health Risk Factor Week

April 6, 2010

"The traditional assay used to measure potency of inactivated influenza vaccines is a single-radial immunodiffusion (SRID) assay that utilizes an influenza strain-specific antibody to measure the content of virus hemagglutinin (HA) in the vaccine in comparison to a homologous HA reference antigen. Since timely preparation of potency reagents by regulatory authorities is challenging and always a potential bottleneck in influenza vaccine production, it is extremely important that additional approaches for reagent development be available, particularly in the event of an emerging pandemic influenza virus."

"An alternative method for preparation of strain-specific antibody that can be used for SRID potency assay is described. The approach does not require the presence or purification of influenza virus, and furthermore, is not limited by the success of the traditional technique of bromelain digestion and purification of virus HA. Multiple mammalian expression vectors, including plasmid and modified vaccinia virus Ankara (MVA) vectors expressing the HAs of two H5N1 influenza viruses and the HA of the recently emerging pandemic H1N1 (2009) virus, were developed. An immunization scheme was designed for the sequential immunization of animals by direct vector injection followed by protein booster immunization using influenza HA produced in vitro from MVA vector infection of cells in culture. Each HA antibody was highly specific as shown by hemagglutination inhibition assay and the ability to serve as a capture antibody in ELISA. Importantly, each H5N1 antibody and the pandemic H1N1 (2009) antibody preparation were suitable for use in SRID assays for determining the potency of pandemic influenza virus vaccines."

"The results demonstrate a feasible approach for addressing one of the potential bottlenecks in inactivated pandemic influenza vaccine production and are particularly important in light of the difficulties in preparation of potency reagent antibody for pandemic H1N1 (2009) virus vaccines."

The full article can be found at: (F. Schmeisser, et. al., "An alternative method for preparation of pandemic influenza strain-specific antibody for vaccine potency determination". *Vaccine*, 2010;28(12):2442-9). Link not available.

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## **DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC ASSAY SPECIFICALLY DETECTING PANDEMIC H1N1 (2009) INFLUENZA VIRUS**

Medical Imaging Week

April 3, 2010

"The pandemic caused by a new type of influenza virus, pandemic H1N1 (2009) influenza virus A (AH1pdm), has had a major worldwide impact. Since hemagglutinin (HA) genes are among the most specific genes in the influenza virus genome, AH1pdm can be definitively diagnosed by viral gene analysis targeting the HA genes."

"This type of analysis, however, cannot be easily performed in clinical settings. While commercially available rapid diagnosis kits (RDKs) based on immunochromatography can be used to detect nucleoproteins (NPs) of influenza A and B viruses in clinical samples, there are no such kits that are specific for AH1pdm. We show here that an RDK using a combination of monoclonal antibodies against NP can be used to specifically detect AH1pdm. The RDK recognized AH1pdm virus isolates but did not recognize seasonal H1N1 and H3N2 and influenza B viruses, indicating that the specificity of the RDK is 100%. A parallel comparison of RDK with a commercial influenza A/B virus kit revealed that both types of kits had equal sensitivities in detecting their respective viruses. Preliminary evaluation of clinical samples from 5 individuals with PCR-confirmed human AH1pdm infection showed that the RDK was positive for all samples, with the same detection intensity as that of a commercial influenza A/B virus kit."

The full article can be found at: (T. Miyoshi-Akiyama, et. al., "Development of an immunochromatographic assay specifically detecting pandemic H1N1 (2009) influenza virus". *Journal of Clinical Microbiology*, 2010;48(3): 703-8). Link not available.

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