

Label-free Real-time Cell Based Assay System for Evaluating H1N1 Vaccination Success

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Since swine-origin influenza A H1N1 virus was identified in Mexico in March, 2009, it spread throughout the world, prompting the World Health Organization (WHO) to raise the pandemic alert to level 6 of 6 on June 11, 2009. Vaccination is the most effective measure to control the spread of the virus and to reduce associated morbidity and mortality. In response to the pandemic, the vaccines against the virus strain A/California/07/2009 (H1N1) have been developed and were approved for vaccination in China since September, 2009. The seed virus was prepared from reassortant vaccine virus strains which were recommended by the WHO, and obtained from the Chinese Food and Drug Administration. The vaccines were manufactured in embryonated chicken eggs according to the same standard techniques that are used to produce the trivalent inactivated vaccine against seasonal influenza. In brief, the virus was propagated in embryonated chicken eggs, harvested from the egg cultures, inactivated with the use of formaldehyde, concentrated, purified and further sterilized.

How to evaluate the success of vaccination becomes the key to ensuring the establishment of population protection and preventing further spread of the infection of

H1N1 virus. The hemagglutination inhibition (HI) assay or microneutralization (MN) assay has been used as the standard methods to indicate seroconversion and evaluate immunoprotection against H1N1 virus after the vaccination. Although a good correlation and specificity have been reported between HI or MN activity and neutralizing activity against H1N1 virus, the definitive diagnosis typically requires end point plaque reduction neutralization test (PRNT) and the demonstration of a four fold difference in titer between competing viruses. The low assay

throughput, high exposure to the life threatening virus, and time consuming of PRNT make the neutralizing antibody detection hardly to be adopted in evaluation of H1N1 vaccination in human population.

An electronic impedance-based cell detection system was applied for fast and quantitative detection of neutralizing antibody activities against H1N1 virus in real time. The system, called real time cell analysis (RTCA) system (xCELLigence, Roche Applied Sciences) was developed using a

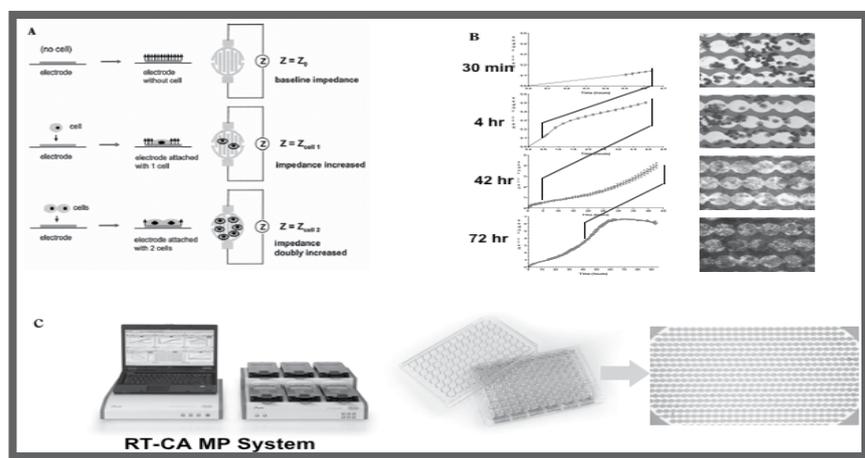


Figure 1. Electronic impedance-based cell detection and RTCA system. A. the principle of impedance detection of cells cultured on the sensor electrode with time. B. monitoring of cell attachment, spreading, growth and stationary states on the sensor in real time. The left panel shows the CI curves representing cell status on the sensor electrodes (the images on right panel). C. the RTCA system, which includes the analyzer, the control unit (computer and software), and the electronic sensor plate (E-plate). To assay samples, the cells are cultured in the E-plates and the cell growth or cell death induced by chemical compounds or virus infection such as H1N1 can be dynamically monitored in real time. The data acquisition is fully automated.

[Feature]

novel electrical impedance sensor array, allowing for quantitative detection of living cells cultured on gold microelectrodes within the glass substrates integrated in the bottom of 96-well microtiter plates (Figure 1). The electronic impedance of an electrode is primarily determined by the ion environment both at the electrode/solution interface and in the bulk solution. Upon the application of an electrical field, ions undergo field-directed movement and concentration-gradient driven diffusion, leading to frequency dependent impedance dispersion. The presence of the cells will affect the local ionic environment at the electrode/solution interface, leading an increase in the electrode impedance. The more cells there are on the electrodes, the larger the electrode impedance (Figure 1). Furthermore, the impedance also depends on the extent to which cells attach to the electrodes. For example, if cells spread, there will be a greater cell/electrode contact area, resulting in larger impedance. Thus, cell biological status including cell viability, cell number, cell morphology and cell adhesion will all affect the measurement of electrode impedance that is reflected by cell index (CI) on the RTCA system. Therefore, a dynamic pattern of a given CI curve may indicate sophisticated physiological and pathological response of the living cells to a given toxic compound or virus infection. In the cells infected with H1N1 virus, the cytopathic effect (CPE) can be quantitative monitored in real time on the RTCA system, and such H1N1-induced CPE can be inhibited or the onset of the CPE can be delayed in the presence of neutralizing antibody against H1N1 after vaccination (Figure 2).

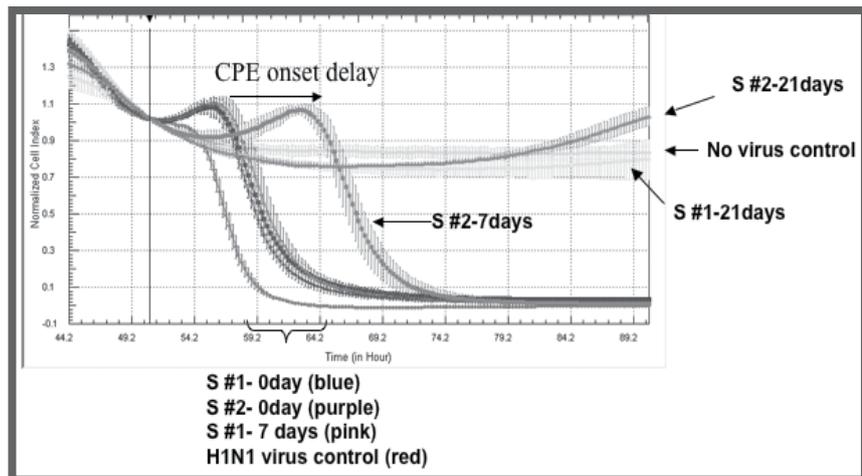


Figure 2. Neutralizing antibody activity measurement in H1N1 vaccinated human subjects using real time monitoring of virus-induced CPE on RTCA system. Samples of two human subjects from different days after vaccination were tested for neutralizing activity against H1N1 virus challenge. In samples from day 0 (before vaccination), no protection can be seen showing the CPE onset similar to virus control. Sample #2 (S#2) at day 7 showed significant delay of H1N1-induced CPE indicating the presence of specific neutralizing antibody against H1N1 virus, and in contrast, Sample #1 (S#1) at day 7 showed no effect on the onset of H1N1 virus-induced CPE. At the day 21, both samples (S#1 and S#2) showed complete protection against H1N1-induced CPE indicating both subjects developed neutralizing antibody against H1N1 virus after vaccination.

Samples of 123 human subjects vaccinated with the virus strain A/California/07/2009 (H1N1) were tested on RTCA system. For each subject, three samples from different times (day 0, day 7 and day 21) were collected and the seroconversion and neutralizing activity against the H1N1 virus were monitored on the RTCA system. On day 7 post-vaccination, 67.48% of vaccinated subjects showed neutralizing antibody positive, and on day 21, 82.11% of subjects showed the neutralizing antibody positive (Sun F, Zheng M, *et al*, unpublished observation). This result indicates that the vaccination is successful. Although, the result obtained from the neutralizing antibody test agreed with that from HI test, the seroconversion rate using neutralizing antibody detection is slightly lower than the seroconversion rate using standard HI test, indicating that the HI activity against H1N1 might not be necessarily related to the

neutralizing activity.

New technologies leading towards a faster and more accurate detection system for life threatening infectious agents are urgently needed. The novel cell based assay system (RTCA system) described here was successfully applied for detecting neutralizing antibody against H1N1 virus in the vaccinated human population. In comparison with existing methods, the RTCA assay offers the advantages of real time, quantitative measurement of entire CPE process and allows for real time monitoring of neutralizing activity and titer. Furthermore, the automatic data acquisition and limited experiment process reduces the possible laboratory exposure to the life threatening infectious agents and thus increases safety.

About the Authors



Dr Hongzhou Lu is the Deputy Director of Shanghai Public Health Clinical Center affiliated to Fudan University, which is the only designated hospital for infectious disease. He also is the Director of Department of Infectious Disease, Shanghai Public Health Clinical Center, and Director of Shanghai HIV/AIDS Diagnosis and Treatment Center. He also is the leader of an expert group in Shanghai HIV/AIDS and 2009 A/H1N1 influenza diagnosis and treatment. Prior to joining Shanghai Public Health Clinical Center, Dr Lu was the Deputy Director and Party Secretary of Department of Infectious Disease, HuaShan Hospital affiliated to Fudan University. Dr Lu has over 7 scientific research prizes, and has published over 180 research articles in China and international journals including the New England Journal of Medicine, AIDS Research and Human Retroviruses and Emerging Infectious Diseases.



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