# Antibody anti H1N1pdm09 detection from Influenza A outbreak cases in Indonesia 2009

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**Abstrak**

***Latar belakang:*** *Infeksi virus Influenza A dapat menyebabkan wabah di seluruh dunia. Wabah virus H1N1 terjadi di Indonesia pada bulan April 2009. Sebanyak 1892 kasus terduga H1N1 dilaporkan antara April dan akhir Desember 2009. Dari 1892 kasus yang terduga, hanya 809 dikonfirmasi terinfeksi virus H1N1. Karena virus strain baru, maka perlu diketahui persentase kasus yang mengembangkan antibodi anti H1N1 setelah infeksi pertama kali.*

***Metode:*** *Penelitian menggunakan sera yang disimpan dari yang dikumpulkan pada tahun 2009 yang disimpan pada-70° C di laboratorium virologi di Lembaga Penelitian dan Pengembangan Kesehatan. Ujia yang digunakan ialah uji HI untuk menentukan kekebalan setelah infeksi Influenza A/H1N1pdm09.*

***Hasil:*** *Hanya sebagian kecil kasus terinfeksi flu A/H1N1pdm09 selama wabah mengembangkan antibodi pelindung anti H1N1pdm09. Dari 205 sera yang diperiksa, 38 kasus (18,5%) mengembangkan antibodi anti H1N1pdm09 dengan HI titer ≥ 1:40.*

***Kesimpulan:*** *Sebanyak 18,5% kasus yang mengembangkan antibodi anti H1N1pdm09 di antara kasus terinfeksi flu A/H1N1pdm09 selama wabah 2009. (**Health Science Indones 2013;1:37-40**)*

***Keywords:*** *Influenza, antibody, pandemic, 2009*


**Abstract**

**Background:** Influenza A virus infection can cause pandemic influenza worldwide. The outbreak of the H1N1 virus occurred in Indonesia in April 2009. A total of 1892 suspected cases of H1N1 were reported between April and the end of December 2009. From 1892 suspected cases, there were only 809 confirmed infected by the H1N1 virus. Since the virus is a new strain, we wanted to know how many cases developed antibody anti H1N1 after the first infection occurred.

**Methods:** We used archived sera which was collected in 2009 and stored at -70oC in the virology laboratory of the National Institute of Health Research and Development. We used the HI assay to determine the immunity after infection of Influenza A/H1N1pdm09 in 2009.

**Results:** Only a small proportion of cases infected during an outbreak of flu A/H1N1pdm09 develop protective antibodies anti H1N1pdm09. Of 205 sera were examined, 38 cases (18.5%) developed anti H1N1pdm09 with HI antibody titer ≥ 1:40.

**Conclusion:** A total of 18.5% of cases develop antibodies anti H1N1pdm09 among A/H1N1pdm09 flu cases during the 2009 outbreak. (***Health Science Indones 2013;1:37-40***)

**Keywords:** Influenza, antibody, pandemic, 2009

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Laboratory-based influenza detection is a system that detects symptomatic patients who seek medical advice and are then appropriately tested and case notifications sent. Measurement of antibodies against influenza A (H1N1pdm09) can be used to assess the spread of population exposure to the virus. The influenza A (H1N1pdm09) is a disease caused by the influenza virus belonging to the family Orthomyxoviridae. Influenza viruses can be divided into three types or genera; A, B and C. Furthermore, influenza virus type A can be divided into many different subtypes based on surface antigens: hemagglutinin (HA) and Neuraminidase (NA). Until now there are found 16 subtypes of HA and 9 NA subtypes.[1] In addition to seasonal influenza A (H3N2) and influenza A (H1Npdm09) virus epidemics, from time to time new influenza A viruses from animal origin are introduced in the human population. When these new viruses are able to spread efficiently among humans, they can cause an influenza virus outbreak since almost all people do not have antibodies against this new virus strains.[2]

In the early 21st century, a new strain of influenza A (H1N1) virus emerged causing an outbreak in the human population. This virus was originally detected in the in Mexico and the United State in March and early April 2009. In June 11, 2009, the World Health Organization announced that the Vigilance Outbreak Influenza has reached the highest phase (phase 6).[3,4] Almost all countries affected by the Influenza A (H1N1pdm09) virus, including Indonesia, became epidemic and according to the influenza virus nomenclature, this novel H1N1 strain was named Influenza A (H1N1pdm09).

The Garten study results indicate that there are dissimilarities between influenza virus A/H1N1pdm09 2009 with the H1N1 influenza virus that circulated earlier.[5] The previous study found that the antigenic characteristics of the influenza A (H1N1pdm09) virus circulating in Indonesia matched the A/California/07/2009 strain.[6]

A total of 1892 suspected cases of influenza A (H1N1pdm09) were reported between April 2009 and the end of December 2009 in Indonesia. The confirmation examination conducted at the Center for Biomedical and Basic Technology of Health was done with realtime RT-PCR according to WHO guidelines using throat and nasal swabs. 890 out of 1892 suspected cases were confirmed positive for infection by Influenza A (H1N1pdm09). Twelve of these cases were fatal. On August 10, 2012, WHO declared that during the outbreak of Influenza A (H1N1pdm09) 206 countries reported laboratory confirmation of the Influenza A (H1N1pdm09).[4]

Since it was a very short time that the outbreak occurred, we wondered about the development of antibody anti influenza A (H1N1pdm09) among the human cases in Indonesia. This study aimed to determine the immunity status of the confirmed cases after the first infection occurred by detection of antibody anti influenza A (H1N1pdm09).

## METHODS

### Samples collection

During the outbreak of Influenza A (H1N1pdm09) in Indonesia, beginning in April 2009, all specimens such as throat and nasal swabs and sera were sent from many hospitals in Indonesia to the Virology Laboratory, National Institute of Health Research and Development as the reference laboratory in Indonesia. Patient swabs were tested by realtime RT-PCR for influenza A (H1N1pdm09) detection, and the sera was stored in the deep freezer until used for the further examination. A total of 205 archived sera out of 890 confirmed influenza A (H1N1pdm09) cases were tested in this study.

### Laboratory examination

To determine the antibody anti H1N1, we used Hemagglutination Inhibition assay (HI) and influenza A (H1N1pdm09) with A/California/07/2009 strain used as the antigen against the sera. A total of 20 ul of serum was incubated with 60 ul respirator destroying enzyme (RDE) at 370 C overnight. Then using V 96 well plate, 50 ul Phosphate Buffer Saline was put in each well. 50 ul of serum was incubated with RDE in the first well. Two-fold serial dilutions were performed, and 50 ul influenza A (H1N1pdm09) antigen was added to all wells and incubated for 1 hour. 0.5% turkey red blood cells were added lastly.[7] **Titer ≥ 1:40 is considered as a positive** antibody anti influenza A (H1N1pdm09).[8]

## RESULTS

Table 1 showed that there was variation in the antibody titer against the antigen A/H1N1pdm09. Among the 205 sera examined, 50 cases showed antibody anti influenza A (H1N1pdm09) with an antibody titer range from ≤ 10 to 640. Most of the cases (75.6%) had low titers. Negative (titers of ≤ 1:20) antigen influenza A (H1N1pdm09) were 81.5%. Therefore, those who had positive antigen influenza A (H1N1pdm09) were 18.5%.

Table 1.  The results of antibody titers against the antigen influenza A (H1N1pdm09)

| Antibody anti influenza A | Titer | Cases (n=205) | | |
|---|---|---|---|---|
| | | n | % | Sub total |
| Negative | < 10 | 155 | 75.6 | 81.5 |
| | 20 | 12 | 5.9 | |
| Positive | 40 | 12 | 5.9 | 18.5 |
| | 80 | 13 | 6.3 | |
| | 160 | 6 | 2.9 | |
| | 320 | 1 | 0.5 | |
| | 640 | 6 | 2.9 | |

## DISCUSSIONS

Influenza A (H1N1pdm09) outbreak occurred in almost all countries in the year 2009. It was a tough challenge for the implementation of the surveillance system and quick handling of the case. By this antibody detection, the degree of immunity from influenza A (H1N1pdm09) cases is known. The data of the Hemagglutination Inhibition (HI) assay could be analyzed more completely, if the results were associated by the age of patient, history of disease, vaccination records and geographic data similar to the study which had been done in Shandong, China. The results of  their seropositive rate for influenza A (H1N1pdm09) antibodies was 25,9% with most cases detected in the 0-5 years age range group and also in the elderly group aged $\geq$ 60 year old.[8] Similar findings were made in Italy.   The highest number of cases with an antibody response for influenza A (H1N1pdm09) were children with a hospitalized history.[10]

Referring to the results of this study, clearly elaborate antibody response in the influenza A (H1N1pdm09) cases, a seropositive rate of 18.6% was observed. If this result is compared with the seropositive rate in the U.S. which was 20.2%, it means that the incidence of cases in Indonesia with influenza A (H1N1pdm09) was almost the same as the incidence of cases in America.[9] In the other countries in the southern hemisphere, such as Australia, New Zealand and Singapore, the post outbreak seropositivity proportion was 17.5% in Singapore. Australia and NZ ranged from 22.1% to 32.8%. The seropositivity in Indonesia is consistent with Singapore as tropical regions show lower seropositivity compared with subtropical regions. These findings might be explained that the transmission of influenza increased in cold temperatures.[11]

Immunity cases in Indonesia influenza A (H1N1pdm09) formed after infection or may have come from previous exposure to influenza A (H1N1pdm09). Furthermore, not all cases directly produce antibodies after infection.

However, this study has limitations. First, serum samples used had been stored since 2009 and had been examined in 2011. So, there is the possibility of antibody levels having decreased due to storage time. Second, all the samples came from an outbreak influenza A (H1N1pdm09), and there was limited data and records on the patents. Therefore, the immune status against influenza A (H1N1pdm09) cannot be analyzed since we did not have the baseline data and the test results could not be linked with the disease incubation period of the patients. Third, there was limited serum for the seroconversions since there was no follow up of the patients after they recovered from the illness and left the hospitals. The seroconversions levels are important to show the immunology status of the patients.  Fourth, according to laboratory tests, HI assay is not the most sensitive examination for detecting antibodies. It needs further laboratory tests with more sensitive detection such as the neutralization assay.

## Conclusions

A total of 18.5% of cases develop antibodies anti H1N1pdm09 among A/H1N1pdm09 flu cases during the 2009 outbreak.

## Acknowledgments

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