Appendix 1: Laboratory methods for hemagglutination inhibition assay used to detect IgG antibodies against the pandemic (H1N1) 2009 virus

The A/California/04/2009 H1N1 virus isolate used in this assay was kindly provided by the Canadian National Microbiology Laboratory. Virus stocks were prepared at the Cadham Provincial Laboratory according to standard procedure.[1] Briefly, 1:10 serial dilution of the virus stock in primer binding site was prepared. Ten-days-old embryonated chicken eggs were inoculated with 100 μL of one of the three virus dilutions (neat, 1:10 and 1:100). The eggs were incubated at 37°C for three days and then chilled at 4°C overnight. The allantoic fluid was collected using sterile 10-mL pipettes; the virus was aliquoted in 1-mL aliquots and stored frozen at –70°C.

Hemagglutination inhibition assay (HIA)

HIA was performed according to a widely used WHO protocol.[1] Sera were treated with receptors destroying enzymes and hemadsorbed on guinea pig red blood cells. A 1:10 dilution of the serum specimens were prepared followed by 1:2 serial dilutions in 25 μL PBS in a 96 microliter plates U bottom. Subject specimens were diluted in rows B–F of the microliter plate. Twenty five μL of PBS containing four hemagglutination units of the H1N1 California strain virus were added to each well. Row A of the microliter plate was saved as a control, where 25 μL of uninfected allantoic fluid was added to each well. The plate was incubated for 30 minutes at room temperature, then 0.8% guinea pig red blood cells in 50 μL was added to each well and incubated for one hour at room temperature. The serum titre was expressed as the reciprocal of the highest serum dilution where hemagglutination was inhibited.

Reference

1. Webster R, Cox N, Stohr K. *WHO manual on animal influenza diagnosis and surveillance*. Geneva (Switzerland): World Health Organization; 2002.

Appendix to: Mahmud SM, Becker M, Keynan Y, et al. Estimated prevalence of pandemic (H1N1) influenza among pregnant women during the first wave of the 2009 pandemic. *CMAJ* 2010.