

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Greenberg ME, Lai MH, Hartel GF, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. *N Engl J Med* 2009;361:2405-13. DOI: 10.1056/NEJMoa0907413.

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METHODS

Study Site

The study was conducted at CMAX, an independent clinical research facility located in Adelaide, Australia. The site has completed studies that complied with registration requirements in Australia, the United States, Canada, Europe and Japan.

Exclusion Criteria

Participants with confirmed or suspected cases of 2009 H1N1 were excluded on the basis of the following: history of confirmed infection with 2009 H1N1; history of an unexplained acute febrile respiratory illness since May 01, 2009; history of prophylactic antiviral medication use against suspected 2009 H1N1 infection.

Vaccine

The vaccine was prepared from influenza virus propagated in the allantoic fluid of embryonated chicken eggs. After harvest, the virus was purified in a sucrose gradient, inactivated, disrupted with detergent, purified and suspended in a phosphate buffered isotonic solution to produce a purified "split virion" vaccine.

At the time of formulation of the vaccine the single radial immunodiffusion (SRID) potency reagents were not available; therefore, a surrogate potency assay was used. The potency of the

vaccine was assigned by calculation of a theoretical potency value using established protein analysis techniques. When the standard reagent became available for SRID testing, the true vaccine potency was assigned. The assigned lower fiducial limit of the vaccine was 60.9 µg/mL (actual value 63.8 µg/mL) corresponding to a nominal potency of 60 µg/mL.

Solicited Adverse Event Intensity Grading Scale

The intensities of solicited adverse events were graded on a four-point scale (none, mild, moderate, severe).

Table 1: Solicited Local Adverse Event Grading Scale

Injection Site Reaction	Intensity Grading			
	None	Mild	Moderate	Severe
Pain	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Tenderness	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Redness (erythema)	< 25 mm	25 to 50 mm	51 to 100 mm	> 100 mm
Induration / swelling	< 25 mm and no functional effect	25 to 50 mm or did not interfere with activity	51 to 100 mm or interfered with activity	> 100 mm or prevented daily activity
Ecchymosis (bruising)	< 25 mm and no functional effect	25 to 50 mm or did not interfere with activity	51 to 100 mm or interfered with activity	> 100 mm or prevented daily activity

Table 2: Solicited Systemic Adverse Event Grading Scale

Symptom	Intensity Grading			
	None	Mild	Moderate	Severe
Fever	< 37.7°C (< 99.9°F)	≥ 37.7°C to < 38.0°C (≥ 99.9°F to < 100.4°F)	≥ 38.0°C to < 39.0°C (≥ 100.4°F to < 102.2°F)	≥ 39.0°C (≥ 102.2°F)
Headache	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Malaise	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Myalgia	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Chills	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Nausea	None	Did not interfere with activity	Interfered with activity	Prevented daily activity
Vomiting	None	Did not interfere with activity	Interfered with activity	Prevented daily activity

Adverse Events of Special Interest

We monitored the following adverse events of special interest:

Nervous System Disorders:

Bell's palsy

Seizure / convulsion

Encephalitis / encephalomyelitis

Transverse myelitis

Guillain-Barré syndrome

Optic neuritis

Immune System Disorders:

Anaphylaxis

Serum sickness

Corneal graft rejection

Other Disorders:

Oculorespiratory syndrome

Thrombocytopenia

Stevens Johnson syndrome /
toxic epidermal necrolysis

Vasculitis

Assay Methods

Assays were conducted by Focus Diagnostics, Incorporated (Cypress, CA, US) according to standard operating procedures. Focus Diagnostics is certified by the Clinical Laboratory Improvement Amendments, the College of American Pathologists and the International Organization for Standardization. All assays were validated according to international standards^{1,2} to ensure that the performance characteristics of the method met the requirements for use with the A/California/7/2009 NYMC X-179A (H1N1)v virus. To minimize assay variation, sera collected at baseline and 21 days after the first vaccination were re-analyzed together with samples collected 21 days after the second vaccination, and results from this second assay are presented.

Antigen used in the assays was supplied by CSL Limited (A/California/7/2009 NYMC X-179A [H1N1]v-like virus antigen). Reference antiserum to A/California/7/2009 was supplied by the National Institute for Biological Standards and Control (NIBSC). The NIBSC also supplied reference antisera to strains contained in the 2009 southern hemisphere seasonal influenza vaccine (A/Brisbane/59/2007 [H1N1], A/Brisbane/10/2007 [H3N2] and B/Florida/4/2006), and these were used as control samples during the assay validation process.

Hemagglutination-Inhibition Assay

Hemagglutination-inhibition assays were performed using turkey red blood cells. Serum samples were treated with receptor destroying enzymes to inactivate nonspecific inhibitors. If necessary, nonspecific agglutinins were removed by absorption to red blood cells. Hemagglutination-inhibition assays were performed in triplicate for each sample, with a starting dilution of the treated serum of 1:5.

The hemagglutination-inhibition assay validation procedure included an evaluation of the accuracy, specificity, sensitivity, precision and linearity of the assay. In particular, the validation included an analysis of cross-reactivity to ensure specificity for the A/California/7/2009 NYMC X-179A (H1N1)v virus. Reference antisera included A/Brisbane/59/2007, A/Brisbane/10/2007 and B/Florida/4/2006. The geometric mean titer obtained with A/California/7/2009 was at least four-fold higher than with other reference antisera, indicating that the assay was specific for A/California/7/2009. Therefore, only the specific reference antiserum and the negative reference antiserum were used as controls for the A/California/7/2009 during clinical testing.

Microneutralization Assay

Influenza virus microneutralisation assay was based upon the inhibition of cytopathic effect formation in Madin-Darby Canine Kidney (MDCK) cells. Serial diluted sera (in duplicate from 1:10 to 1:5120) were pre-incubated with a standardized amount of virus (100 TCID₅₀ / 50 µL). MDCK cells were then added and the plates incubated at 37°C overnight. Cells were then fixed and the presence of virus detected with an anti-nucleoprotein antibody using an enzyme-linked immunosorbent assay. The virus neutralizing antibody endpoint titer was determined using a formula that calculated the midpoint optical density of uninfected cells and virus-infected (without neutralization) cells. The 50% neutralizing titer (NT₅₀) for each sample was calculated by $NT_{50} = 5 \times 2^{(N/2 + 0.5)}$, where N was the total number of neutralized wells.

RESULTS

Table 3: Immune Response by Baseline Serostatus after the First and Second Dose of the H1N1 Vaccine, as Measured on Hemagglutination-Inhibition Assay and Microneutralization Assay.

Assay, Dose, and Baseline Serostatus	Subjects with Seroconversion or Significant Increase in Titer		Factor Increase in Geometric Mean Titer	
	After First Dose	After Second Dose	After First Dose	After Second Dose
Hemagglutination-inhibition assay				
15- μ g dose				
Baseline titer < 1:10	92.9 (80.5-98.5)	95.2 (83.8-99.4)	27.1 (18.1-40.6)	36.3 (25.6-51.5)
Baseline titer \geq 1:10	64.1 (52.4-74.7)	74.7 (63.3-84.0)	7.6 (5.4-10.7)	10.1 (7.4-13.8)
30- μ g dose				
Baseline titer < 1:10	83.7 (70.3-92.7)	93.6 (82.5-98.7)	35.8 (22.7-56.5)	47.2 (32.3-68.9)
Baseline titer \geq 1:10	80.0 (68.7-88.6)	86.8 (76.4-93.8)	12.8 (8.9-18.3)	14.3 (10.4-19.6)
Microneutralization assay				
15- μ g dose				
Baseline titer < 1:10	77.4 (65.0-87.1)	91.7 (81.6-97.2)	38.9 (23.7-63.8)	58.0 (39.7-84.8)
Baseline titer \geq 1:10	77.6 (64.7-87.5)	82.5 (70.1-91.3)	15.4 (9.6-24.5)	17.4 (11.5-26.5)
30- μ g dose				
Baseline titer < 1:10	74.0 (62.4-83.5)	87.3 (77.3-94.0)	50.2 (29.9-84.5)	67.9 (45.0-102.2)
Baseline titer \geq 1:10	78.3 (63.6-89.1)	77.3 (62.2-88.5)	27.3 (14.7-50.8)	25.3 (13.8-46.1)

Table 4: Percentage of 240 Subjects Who Reported Having a Solicited Local or Systemic Adverse Event within 7 Days after Receiving the First or Second Dose of the H1N1 Vaccine

Adverse Event	Mild		Moderate		Severe		All Grades	
	15 µg	30 µg	15 µg	30 µg	15 µg	30 µg	15 µg <i>(95% confidence interval)</i>	30 µg <i>(95% confidence interval)</i>
Solicited local event								
Any	48.3	53.3	2.5	8.3	0.0	0.0	50.8 (41.6-60.1)	61.7 (52.4-70.4)
Pain	24.2	27.5	1.7	2.5	0.0	0.0	25.8 (18.3-34.6)	30.0 (22.0-39.0)
Tenderness	38.3	49.2	0.8	5.8	0.0	0.0	39.2 (30.4-48.5)	55.0 (45.7-64.1)
Redness	1.7	1.7	0.8	1.7	0.0	0.0	2.5 (0.5-7.1)	3.3 (0.9-8.3)
Induration	10.8	11.7	0.0	0.8	0.0	0.0	10.8 (5.9-17.8)	12.5 (7.2-19.8)
Ecchymosis	6.7	6.7	0.0	0.8	0.0	0.0	6.7 (2.9-12.7)	7.5 (3.5-13.8)
Solicited systemic event								
Any	35.0	41.7	10.0	17.5	1.7	1.7	46.7 (37.5-56.0)	60.8 (51.5-69.6)
Any related	26.7	31.7	3.3	11.7	1.7	0.0	31.7 (23.5-40.8)	43.3 (34.3-52.7)
Fever	3.3	1.7	1.7	2.5	0.0	0.0	5.0 (1.9-10.6)	4.2 (1.4-9.5)
Headache	29.2	34.2	5.8	9.2	0.0	0.0	35.0 (26.5-44.2)	43.3 (34.3-52.7)
Malaise	14.2	22.5	2.5	8.3	1.7	0.0	18.3 (11.9-26.4)	30.8 (22.7-39.9)
Myalgia	18.3	17.5	2.5	5.8	0.8	0.8	21.7 (14.7-30.1)	24.2 (16.8-32.8)
Chills	1.7	11.7	0.0	1.7	0.0	0.0	1.7 (0.2-5.9)	13.3 (7.8-20.7)
Nausea	5.8	6.7	0.8	2.5	0.8	0.8	7.5 (3.5-13.8)	10.0 (5.3-16.8)
Vomiting	0.0	0.0	0.0	1.7	0.0	0.0	0.0 (0.0-3.0)	1.7 (0.2-5.9)

Table 5: Percentage of 240 Subjects Who Reported Having an Unsolicited Adverse Event within 21 Days after Receiving the First or Second Dose of the H1N1 Vaccine

Adverse Event	Mild		Moderate		Severe		All Grades	
	15 µg	30 µg	15 µg	30 µg	15 µg	30 µg	15 µg	30 µg
Any unsolicited event	25.0	20.8	17.5	20.0	1.7	5.0	44.2	45.8
Any related unsolicited event	4.2	5.8	1.7	3.3	0.8	0.0	6.7	9.2
Headache	10.8	12.5	5.0	8.3	0.8	0.0	16.7	20.8
Oropharyngeal pain	4.2	5.0	0.0	1.7	0.0	0.0	4.2	6.7
Back pain	2.5	0.0	4.2	0.8	0.0	0.8	6.7	1.7
Cough	1.7	3.3	0.0	1.7	0.8	0.0	2.5	5.0
Upper respiratory tract infection	1.7	1.7	0.8	0.0	0.8	1.7	3.3	3.3
Diarrhea	2.5	0.8	1.7	0.8	0.0	0.0	4.2	1.7
Dysmenorrhea	0.0	2.5	0.0	3.3	0.0	0.0	0.0	5.8
Pyrexia	3.3	0.0	0.0	2.5	0.0	0.0	3.3	2.5
Nasopharyngitis	1.7	0.0	1.7	1.7	0.0	0.0	3.3	1.7
Rhinorrhea	0.0	4.2	0.8	0.0	0.0	0.0	0.8	4.2

Figure 1

Reverse Cumulative Distribution Curves of Antibody Titers in Serum before and 21 Days after a Single Dose of H1N1 Vaccine, According to the Type of Assay.

Shown are levels of antibody titer against the 2009 H1N1 virus on hemagglutination-inhibition (HI) assay before vaccination (Panel A) and after vaccination (Panel B) and in the two age groups in the study (18 to 49 years and 50 to 64 years) (Panels C and D). Also shown are levels of antibody titer against the 2009 H1N1 virus on microneutralization (MN) assay before vaccination (Panel E) and after vaccination (Panel F) and in the two age groups (Panels G and H).

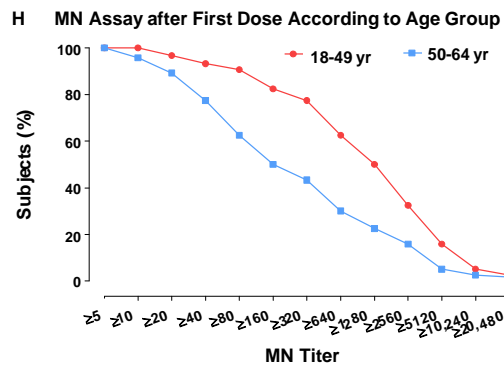
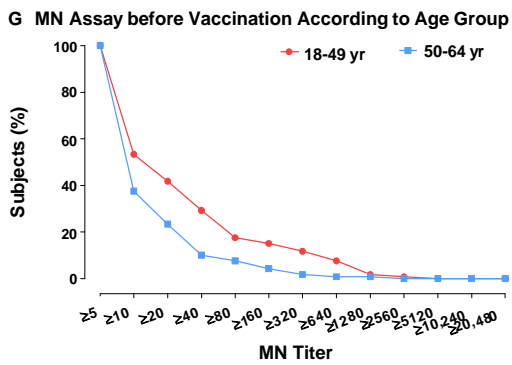
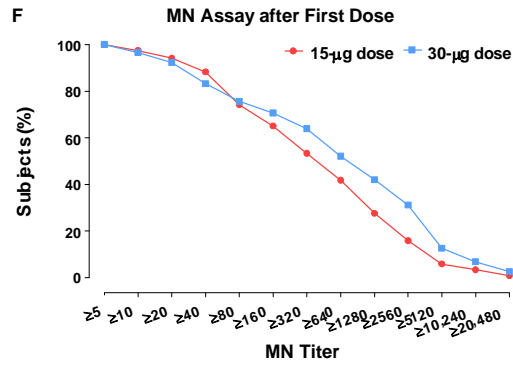
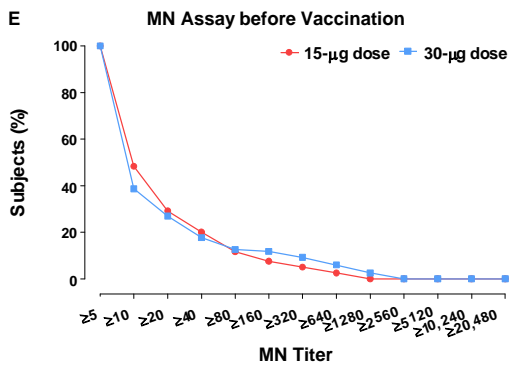
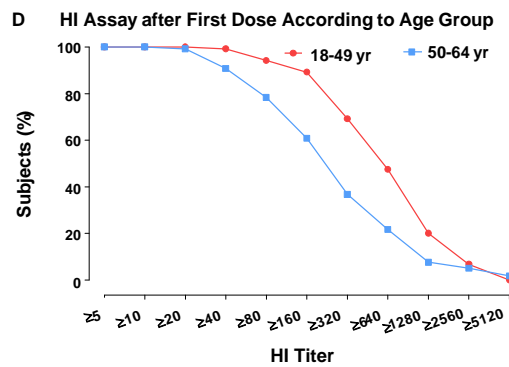
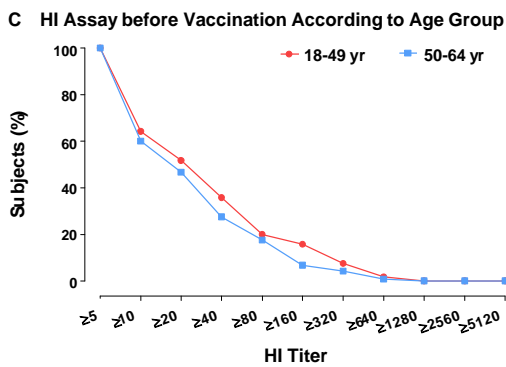
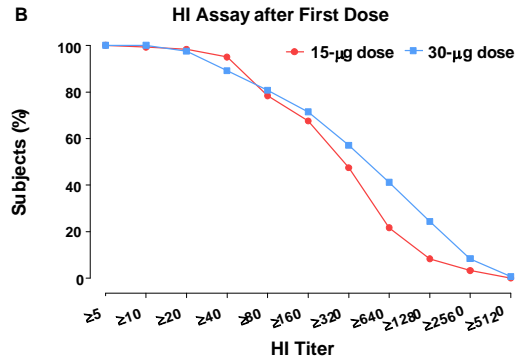
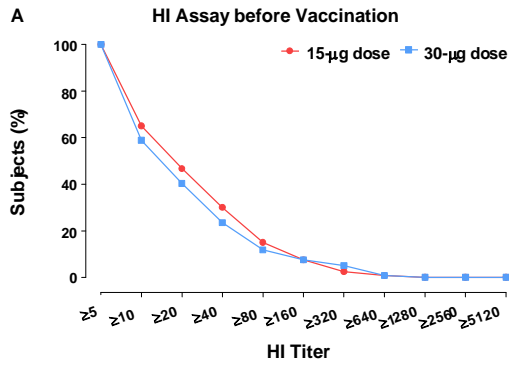
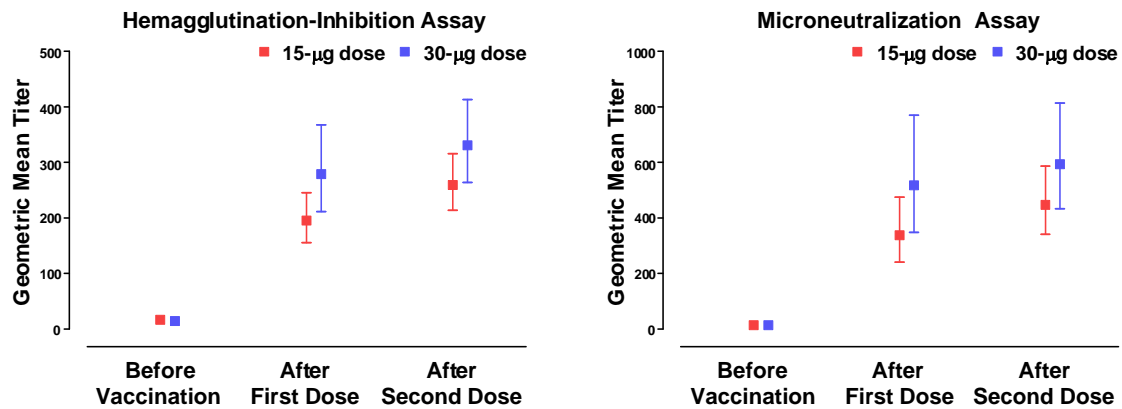


Figure 2

Geometric Mean Titer (with 95% Confidence Intervals) before Vaccination, 21 Days after the First and Second Dose of the H1N1 vaccine, According to the Type of Assay.



REFERENCES

1. ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology. Q2(R1). November 2005.
2. Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine. May 2001.