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# Safety and Immunogenicity of an Inactivated Influenza A/H5N1 Vaccine Given with or without Aluminum Hydroxide to Healthy Adults: Results of a Phase I—II Randomized Clinical Trial

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## Abstract

**Background—**Dose-sparing strategies are being explored for vaccines against pandemic influenza. We evaluated the dose-sparing potential of aluminum hydroxide (AlOH) adjuvant.

**Methods—**A total of 600 healthy subjects (age, 18–49 years) were randomized to receive 2 vaccinations 1 month apart with subvirion inactivated influenza A/H5N1 vaccine containing 7.5, 15, or 45 μg of hemagglutinin (HA), with or without 600 μg of aluminum hydroxide (AlOH), or 3.75 μg of HA, with or without 300 μg of AlOH. Serum specimens were obtained for antibody assays before and 1 month after each vaccination.

**Results—**All formulations were safe. Injection site discomfort was more frequent in groups given vaccines with AlOH. Dose-related increases in antibody responses were noted after both vaccinations ($P < .001$): geometric mean titers of hemagglutination inhibition antibody in vaccines with and without AlOH, respectively, were 5.4 and 5.4 for subjects who received 3.75 μg of HA, 7.7 and 5.3 for those who received 7.5 μg of HA, 8.1 and 8.5 for those who received 15 μg of HA, and 14.8 and 12 for those who received 45 μg of HA. A ≥4-fold increase in titer was observed in 2% and 2% of subjects who received 3.75 μg of HA with or without AlOH, respectively; in 14% and 0% who received 7 μg of HA; in 14% and 13% who received 15 μg of HA; and in 33% and 25% who received 45 μg of HA. Addition of AlOH enhanced responses only for subjects who



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received 7.5 μg of HA, but responses in subjects who received 7.5 μg of HA without AlOH were unexpectedly low.

**Conclusion—**Overall, a meaningful beneficial effect of AlOH adjuvant was not observed.

The emergence of novel influenza A virus strains (including subtype A/H5N1, H7N7, and H9N2 viruses) in human populations in recent years has resulted in a global effort to develop candidate vaccines, particularly those active against highly pathogenic influenza A/H5N1 viruses. Recent data suggest that vaccines containing greater-than-expected doses of inactivated influenza A/H5N1 are required to elicit detectable immune responses in a majority of subjects: 2 vaccinations with an inactivated subvirion vaccine containing 90 μg of hemagglutinin (HA) each were necessary to elicit antibody responses in less than half of the subjects [1]. Although vaccination with vaccine containing large doses of inactivated influenza virus is safe and enhances immunogenicity among healthy young adults and older persons [2], there is concern is that use of such large doses for mass vaccination will not be practical during a pandemic, especially if multiple inoculations are required, given the available worldwide capacity for influenza vaccine production. Therefore, dose-sparing approaches are being pursued [3].

Previous studies have shown that inclusion of an adjuvant can enhance immune responses to inactivated influenza A/H5N1 vaccines. IVVs formulated with MF59, a squalene-containing oil-in-water emulsion, are licensed for use in Europe [4]. However, the only type of adjuvant licensed for use in the United States is mineral-containing adjuvant, such as aluminum hydroxide (AlOH). AlOH is a common adjuvant used in many vaccines around the world. In a recent clinical trial, a candidate whole-virus influenza A/H2N2 vaccine containing as little as 2 μg of HA adjuvanted with AlOH was as immunogenic as a subvirion vaccine containing 15 μg of HA without AlOH [5]. Other investigators have recently explored the potential for aluminum-containing compounds to confer adjuvant effects for subvirion and whole-virus inactivated influenza A/H5N1 vaccines [6–11]. Results of these trials have been variable, and nonadjuvanted formulations were not always compared with adjuvanted formulations. In view of the dose-sparing potential of aluminum-containing vaccine formulations, we compared the reactogenicity and immunogenicity of a monovalent subvirion influenza A/H5N1 vaccine, formulated with or without AlOH, that included different doses of HA in healthy adults. In one previous study evaluating AlOH adjuvant with a very similar inactivated split-product H5 vaccine, there was no significant enhancement of the immune response with the addition of 600 μg of AlOH [6]. To confirm and extend these results, we performed an additional study in a larger number of subjects that involved a broader range of vaccine doses.

## SUBJECTS, MATERIALS, AND METHODS

### Vaccine doses

Inactivated subvirion influenza A/H5N1 vaccine was prepared using the A/Vietnam/1203/04 × A/PR/8/34 reassortant virus, derived by means of reverse-genetics techniques [1]. Three doses of vaccine containing 7.5, 15, or 45 μg of HA per 0.5 mL and one 0.25-mL dose of vaccine containing 3.75 μg of HA were formulated with or without AlOH adjuvant. The AlOH content was 600 μg for each 0.5-mL dose and 300 μg for the 0.25-mL dose (sanofi Pasteur).

### Study design and subjects

We conducted a multicenter, randomized, double-blind, placebo-controlled clinical trial. Written informed consent was obtained from potential subjects before screening. Healthy nonpregnant adults between the ages of 18 and 49 years who had no known allergy to

vaccine components (including eggs) and who had not previously received an influenza A/H5 vaccine were considered eligible. The study was conducted in accordance with protocols approved by institutional review boards at the participating study sites.

## Study procedures

Eligible subjects were randomly assigned to receive 2 vaccinations with vaccine containing identical levels of HA in the deltoid muscle ~28 days apart. Vaccinations were administered by personnel who were not involved in the assessment of responses after vaccination. Subjects were observed for 30 min after each vaccination. For 7 days after each vaccination, subjects recorded their oral temperature and the presence and severity of injection site symptoms (pain, tenderness, redness, and swelling) and systemic symptoms (feverishness, malaise, myalgia, headache, and nausea) on a memory aid. Subjects were seen in the clinic on days 2 and 8 after each vaccination, at which time their memory aids were reviewed by study staff. Twenty-eight days after each vaccination and 6 months after the second vaccination, the interim medical history of each subject was reviewed. Blood samples for antibody assays were collected before and 1 month after each vaccination and 6 months after the second vaccination.

The severity of solicited adverse events (AEs) was scored on a scale from 0 to 3, where 0 was defined as no symptom, 1 as a mild symptom that did not interfere with activity, 2 as a moderate symptom that interfered with activity, and 3 as a severe, incapacitating symptom. Injection site redness and swelling were graded according to their diameters, as follows: 0, no redness or swelling (diameter, <0.5 cm); 1, small diameter (0.5–4.9 cm); 2, medium diameter (5–10 cm); and 3, large diameter (> 10 cm). Serious AEs (SAEs) were defined as life-threatening AEs, or AEs that resulted in significant or persistent disability, hospitalization, or death. All reported AEs that occurred during the first 2 months were recorded, as were all reported SAEs that occurred during the entire study period.

## Laboratory assays

Hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays were performed at Southern Research Institute as described previously [1], with the exceptions that the same starting dilution was defined as 1:10 rather than 1:20 and that samples with negative test results were assigned a titer of 5. Therefore, an HAI antibody titer of 40 in the current study would correspond to an HAI titer of 80 in the study by Treanor et al. [1], Seroresponse was defined as an increase of ≥4-fold in antibody titer after vaccination (if antibody was detectable in the prevaccination sample) or an increase in antibody titer from <10 before vaccination to ≥40 after vaccination [12].

## Statistical considerations

The primary objectives of the study were to determine the dose-related safety of subvirion-inactivated H5N1 vaccine adjuvanted with AlOH in healthy adults and to assess the potential for AlOH to enhance immune responses to an inactivated H5N1 vaccine in healthy adults. The prespecified primary reactogenicity end points were the frequencies and severities of AEs or SAEs solicited in the clinic and via memory aids and periodic targeted physical assessment. The primary immunogenicity end points included the proportion of subjects in each group who achieved a serum HAI or Neut antibody titer of ≥40 against influenza A/H5N1 virus 28 days after receipt of the second vaccination and the geometric mean titer (GMT) and frequency of significant HAI and Neut antibody responses in each group 28 days after receipt of the second vaccination. In multivariate analyses, the analytic model used to evaluate differences between groups for injection site/systemic reactogenicity and 4-fold increases in antibody titer (i.e., dichotomous outcomes, such as with pain or without pain) was a logistic regression model. The analytic model used to assess for

differences in GMTs (continuous outcomes) was a generalized linear model, *P* values were not corrected for multiple comparisons.

Based on the assumption that participants who received non-adjuvanted vaccine with 45 μg of HA would have a response rate of ~40%, the study had a power of >80% to detect an increase of ≥50% in the response rate among those who received adjuvanted vaccine with 45 μg of HA.

## RESULTS

A total of 600 subjects were enrolled between March and May 2006; 574 received 2 inoculations with vaccine, and 570 had serum specimens available for antibody assays after receipt of both vaccinations. Five subjects withdrew after enrollment (3 after receipt of the first vaccination, and 2 after receipt of the second vaccination). Four of these subjects were lost to follow-up, and 1 was unable to attend study visits. Three subjects received the incorrect dose of vaccine for the first or second vaccination and were excluded from safety and immunogenicity analyses. Baseline demographic characteristics of enrolled subjects are shown in table 1. No significant differences in baseline age, sex, or race were noted between the vaccine groups.

### Safety and Reactogenicity

**Safety—**Five SAEs were reported during the study period, none of which was considered to be associated with vaccination: breast cancer; gallstone pancreatitis; acute appendicitis; teno-synovitis; and gastroenteritis requiring hospitalization. All events were reported ≥70 days after receipt of the second vaccination. No deaths were reported.

**Injection site reactogenicity—**Pain and tenderness at the injection site were the most common solicited AEs. The frequencies of injection site tenderness during the week after receipt of each vaccination are shown in figure 1. Most injection site symptoms were mild and peaked in frequency on day 0 or 1. Dose-related increases in the frequencies of injection site pain after the first vaccination were observed regardless of nonadjuvanted ($P < .0001$) or adjuvanted ($P < .07$) vaccine status (data not shown). For each dose of HA, the frequencies of injection site pain and tenderness in groups that received adjuvanted vaccine were significantly higher than those in groups that received non-adjuvanted vaccine ($P < .005$ for all comparisons). After the second vaccination, increases in the frequencies of pain or tenderness were observed only in the groups that received nonadjuvanted vaccine ($P \leq 001$ for both comparisons). The frequencies of pain and tenderness in the groups that received 3.75 μg of HA plus adjuvant and 7.5 μg of HA plus adjuvant were greater than in the groups that received nonadjuvanted vaccine with corresponding HA doses ($P < .01$ for both comparisons). The frequency of injection site pain after the second vaccination in the group that received 15 μg of HA plus adjuvant was less than that after the first vaccination ($P = .04$), and the frequencies of pain and tenderness after the second vaccination in the group that received 45 μg of HA plus adjuvant were less than those after the first vaccination ($P = .0015$ and $P = .0005$, respectively) (data not shown). In logistic regression analyses, increased HA dose, inclusion of adjuvant, and younger age were independently associated with a higher frequency of injection site pain or tenderness after the first and second vaccinations; female sex was associated with a higher frequency of injection site discomfort after the second vaccination only (data not shown).

**Systemic reactogenicity—**No dose-related increases in systemic symptoms were observed in the nonadjuvanted or adjuvanted groups after the first or second vaccination. No significant differences in frequencies of systemic reactions were seen when comparing

adjuvanted and nonadjuvanted groups at any dose after the first or second vaccination, with the exception of the groups that received 15 μg of HA, for which the frequencies of nausea after the first vaccination and headache after the second vaccination were higher among those who received adjuvanted vaccine ($P = .03$ and $P = .04$, respectively) (data not shown). In logistic regression analyses, female sex was associated with a higher frequency of malaise, myalgia, and headache after both vaccinations; younger age was associated with a higher frequency of malaise after the first vaccination; and inclusion of adjuvant was associated with a higher frequency of headache after the second vaccination (data not shown).

### Immunogenicity

**Dose-response relationships after vaccination—**GMTs of serum HAI and Neut antibody before vaccination were similar among all groups (range, 5.0–5.3 for HAI antibody and 5.4–5.8 for Neut antibody; $P$ = not significant [data not shown]). Serum antibody responses 1 month after the first and second vaccinations are shown in table 2 and figure 2. After the first vaccination, dose-related increases in the GMTs of serum HAI and Neut antibody were observed for the groups given adjuvanted vaccine ($P < .01$ and $P < .001$, respectively, by analysis of variance); similar dose-related increases in GMTs of serum Neut antibody also were observed for groups given nonadjuvanted vaccines ($P < .001$, by analysis of variance). Dose-related increases in the proportions of subjects with a ≥4-fold increase in HAI antibody titer and the proportions of subjects with an HAI antibody titer of ≥40 after receipt of the first vaccination were observed for the groups given adjuvanted vaccines ($P = .018$ for both comparisons, by the Fisher exact test). Dose-related increases in the proportions of subjects with a ≥4-fold increase in Neut antibody titer and the proportions of subjects with a titer of ≥40 after receipt of the first vaccination were observed for the groups given nonadjuvanted vaccines ($P < .001$ for both comparisons, by the Fisher exact test). Significant dose-response relationships for serum HAI and Neut antibody responses (GMTs, the proportions of subjects with a ≥4-fold increase in titer, and the proportions of subjects with a titer of ≥40) were observed after receipt of the second vaccination, regardless of the inclusion of adjuvant ($P < .001$ for all comparisons).

**Effect of AlOH on immune responses—**No significant differences in GMTs of serum HAI or Neut antibody were observed after the first vaccination between adjuvanted and nonadjuvanted groups given similar doses of HA, Only the 7.5-μg HA dose was associated with a significant difference in serum antibody response after the second vaccination, with the response among those who received nonadjuvanted vaccine significantly lower than the response among those who received adjuvanted vaccine. No subject given the 7.5-μg dose of HA without adjuvant developed a ≥4-fold increase in HAI titer after the second vaccination, compared with 8 (14%) of 59 subjects given the same HA dose with adjuvant ($P < .01$, by the Fisher exact test). The Neut antibody titer among subjects who received 7.5 μg of HA increased by ≥4-fold in 5 (8%) of 55 subjects who received nonadjuvanted vaccine, compared with 14 (24%) of 59 who received adjuvanted vaccine ($P = .045$, by the Fisher exact test). In regression analyses, increased HA dose, female sex, and younger age were associated with higher serum HAI and Neut antibody response frequencies and/or GMTs after the second vaccination; a significant effect of adjuvant was noted for GMTs of serum Neut antibody (data not shown).

## DISCUSSION

Aluminum-containing adjuvants have been shown to enhance antibody responses to a number of protein antigens. Several groups have previously investigated the effect of adsorbing influenza vaccines other than those targeting influenza A/H5N1 virus on

aluminum-containing adjuvants. Hennessy and Davenport [13] demonstrated that an aluminum phosphate-adsorbed vaccine elicited higher mean antibody levels following booster vaccination of infants, compared with infants given aqueous vaccines; however, overall response frequencies were similar. In contrast, Davenport et al. [14] observed no difference in previously primed adults. Gerth and Mok-Hsu [15] reported that an AlOH-adsorbed subvirion vaccine elicited a higher frequency of injection site reactions than did aqueous vaccine, but they were unable to demonstrate significant enhancement of antibody responses. Conversely, Pressler et al. [16] compared an aluminum oxide-adjuvanted influenza vaccine and its aqueous counterpart Modest enhancement in antibody responses was reported among subjects given adsorbed vaccine. However, the vaccine was incompletely described, and no statistical comparisons were provided. Potter et al. [17] were unable to demonstrate an adjuvant-associated effect. Nicholson et al. [18] reported clinical trials of aqueous and AlOH-adsorbed monovalent A/USSR/77 (H1N1) vaccine. Responses were somewhat greater among unprimed subjects (age, 12–25 years) who were given adsorbed vaccine containing 9 μg of influenza A/H1N1 HA: GMTs of HAI antibody and percentages of subjects with a titer of ≥40 following the second vaccination were 124 and 85%, respectively, for the adjuvanted group (51 subjects) and 75 and 70%, respectively, for the group given aqueous vaccine (29 subjects). No statistical comparisons were provided [18].

AlOH adjuvant has been shown to confer significant antigen-sparing effects in a mouse model of H5N1 vaccination [19]. Dose-sparing approaches are now being evaluated in clinical trials of influenza A/H5N1 vaccines in view of the high levels of antigen required to elicit detectable immune responses. Bresson et al. [6] reported clinical and serologic responses among 300 healthy younger subjects given a subvirion influenza A/H5N1 vaccine containing 7.5, 15, or 30 μg of HA with or without AlOH adjuvant. Immune responses were not significantly improved with the addition of adjuvant, although point estimates suggested that response rates were modestly higher for the adjuvanted formulation containing 30 μg of HA and somewhat lower for the adjuvanted formulation containing 7.5 μg of HA, compared with nonadjuvanted formulations at the corresponding HA doses. Bernstein et al. [7] noted that inclusion of AlOH resulted in reduction of immune responses among healthy adults given inactivated vaccine containing 15 or 30 μg of influenza A/H5N1. Nolan et al. [8] noted a modest enhancement in the proportion of subjects who achieved a Neut antibody titer ≥20 after vaccination with inactivated subvirion H5N1 vaccine containing aluminum phosphate adjuvant ($AlPO_4$): 51% and 37% of subjects given 7.5 μg with or without $AlPO_4$, respectively, and 54% and 51% given 15 μg with or without $AlPO_4$, respectively, achieved this titer after 2 vaccinations. Several groups have reported the immunogenicity of whole-virus influenza A/H5N1 vaccines containing AlOH; however, no comparison with nonadjuvanted vaccine was provided [9,10]. Finally, inclusion of AlOH adjuvant in a Vero cell culture–grown whole-virus H5N1 vaccine recently was shown to reduce the immunogenicity of the vaccine [11].

Our results confirm and extend previous observations related to the use of aluminum-containing adjuvants to improve the immunogenicity of IVVs. The frequencies of injection site reactogenicity were increased in groups given AlOH-adjuvanted vaccine, and dose-related increases in the frequencies of injection site discomfort were noted, as observed previously [1,2,6]. Clinically meaningful increases in immunogenicity were not observed when responses among subjects given adjuvanted preparations were compared with responses among subjects given similar doses containing no AlOH. The low response rates among subjects given the nonadjuvanted vaccine containing 7.5 μg of HA were unexpected. Of note, a similar study that used the same vaccine formulations was conducted among persons who were ≥65 years old and found that immune responses to this dose were similar among groups given vaccine with or without adjuvant [20]. By comparison, response rates

among healthy younger adults given 2 doses of a similar nonadjuvanted vaccine containing 90 μg of HA each were recently reported to be 44%, using the new definition of the starting dilution we used for our analyses [21]

In contrast to aluminum-containing adjuvants, oil-in-water emulsions have conferred significant adjuvant effects on candidate pandemic IVVs. Nicholson et al. [22] described the safety and immunogenicity of a purified surface antigen (PSA) vaccine derived from nonpathogenic influenza A/Duck/Singapore (H5N3). Serum HAI and Neut antibody responses were observed in 6 and 8 of 10 healthy adult subjects, respectively, who were given 2 vaccinations with MF 59–adjuvanted vaccine containing 7.5 μg of HA. Nonadjuvanted vaccine was poorly immunogenic. Similar results were reported by Atmar et al. [23], using a vaccine prepared from another potential pandemic virus, A/Hong Kong/97 (H9N2): 2 MF 59-adjuvanted doses containing as little as 3.75 μg of HA stimulated responses in >75% of subjects. The significant adjuvant effect of an oil-in-water adjuvant system on immune responses following vaccination with an inactivated A/H5N1 vaccine resulted in a European Union license application for a prepandemic vaccine earlier this year [24]. More than 80% of healthy adult subjects given two 3.8-μg doses of adjuvanted vaccine responded. As observed in the study by Atmar et al. [23], no dose-response relationships for adjuvanted vaccine were apparent.

The mechanisms by which aluminum-containing adjuvants enhance immune responses are related to the structure of the specific mineral salt, the properties of the adjuvant, and the adsorption mechanism [25]. The reasons for the general failure of AlOH adjuvants to enhance immune responses to influenza virus HA in humans are unknown but may be related to a number of factors, including the strength of adsorption of the HA to the adjuvant, the ratio of antigen to adjuvant, and the interactions of the adjuvanted preparation with interstitial fluid. Combination of an aluminum-containing adjuvant with another type of adjuvant may enhance immune responses; these approaches deserve additional study. On the basis of the variable effects on immunogenicity, dose dependence, and at-best modest effects of AlOH on enhancing the immunogenicity of influenza vaccines, we conclude that alternative dose-sparing approaches must be pursued in the development of vaccines for influenza A/H5N1.

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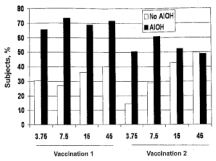


**Figure 1.**
Subjects with injection site tenderness during the week after receipt of inactivated influenza A/H5N1 vaccine with or without aluminum hydroxide (AlOH) adjuvant.

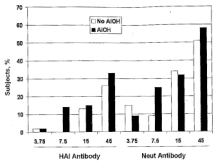


**Figure 2.**
Subjects achieving a serum hemagglutination inhibition (HAI) or neutralizing (Neut) antibody titer of ≥40 after receipt of 2 vaccinations with inactivated influenza A/H5N1 vaccine with or without aluminum hydroxide (AlOH) adjuvant.

**Table 1**

Demographic characteristics of a population vaccinated with inactivated influenza A/H5N1 vaccine, by hemagglutinin (HA) and aluminum hydroxide (AlOH) adjuvant levels.

| | 3.75 μg HA[a] | | 7.5 μg HA | | 15 μg HA | | 45 μg HA | | |
|---|---|---|---|---|---|---|---|---|---|
| **Characteristic** | **300 μg AlOH[a] (*n* = 61)** | **No AlOH (*n* = 59)** | **600 μg AlOH (*n* = 60)** | **No AlOH (*n* = 59)** | **600 μg AlOH (*n* = 61)** | **No AlOH (*n* = 58)** | **600 μg AlOH (*n* = 120)** | **No AlOH (*n* = 119)** | ***P*** |
| Sex no of subjects | | | | | | | | | 76 |
| Male | 23 | 29 | 21 | 27 | 24 | 24 | 55 | 49 | |
| Female | 38 | 30 | 39 | 32 | 37 | 34 | 65 | 70 | |
| Age, mean ± SD, years | 32.6 ± 9.0 | 31.6 ± 9.2 | 30.8 ± 8.2 | 33.7 ± 9.1 | 33.5 ± 8.2 | 32.8 ± 9.4 | 33.4 ± 9.1 | 32.9 ± 9.1 | .61 |
| Race no of subjects | | | | | | | | | 68 |
| White | 46 | 43 | 48 | 47 | 51 | 50 | 100 | 92 | |
| Black | 8 | 7 | 8 | 6 | 7 | 5 | 9 | 15 | |
| Asian | 4 | 5 | 4 | 5 | 2 | 3 | 7 | 4 | |
| Other | 3 | 4 | 0 | 1 | 1 | 0 | 4 | 8 | |

**NOTE.** HA and AlOH levels are per 0.5-mL dose of vaccine, unless otherwise indicated.

[a] Per 0.25-mL dose of vaccine.

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**Table 2**

Serum hemagglutination inhibition (HAI) and neutralizing antibody responses 1 month after the first and second vaccinations with inactivated influenza A/H5N1 vaccine, by hemagglutinin (HA) and aluminum hydroxide (AlOH) adjuvant levels.

| | 3.75 μg HA[a] | | 7.5 μg HA | | 15 μg HA | | 45 μg HA | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **300 μg AlOH[a]** | **No AlOH** | **600 μg AlOH** | **No AlOH** | **600 μg AlOH** | **No AlOH** | **600 μg AlOH** | **No AlOH** |
| HAI antibody | | | | | | | | |
| Geometric mean titer | | | | | | | | |
| After vaccination 1 | 5.5 (4.8–6.2) | 5.4 (4.7–6.2) | 5.5 (5.0–6.0) | 5.5 (4.9–6.1) | 5.3 (4.9–5.9) | 6.5 (5.1–8.3) | 7.6 (6.2–9.3) | 7.3 (5.9–9.1) |
| After vaccination 2 | 5.4 (4.8–6.0) | 5.4 (4.9–6.0) | 7.7 (6.0–9.9) | 5.3 (4.9–5.8) | 8.1 (6.3–10.6) | 8.5 (6.3–11.4) | 14.8 (11.2–19.6) | 12.0 (9.3–15.4) |
| Increase in titer,[b] % of subjects | | | | | | | | |
| After vaccination 1 | 3 | 2 | 0 | 2 | 2 | 7 | 10 | 11 |
| After vaccination 2 | 2 | 2 | 14 | 0 | 14 | 13 | 33 | 15 |
| Neutralizing antibody | | | | | | | | |
| Geometric mean titer | | | | | | | | |
| After vaccination 1 | 6.2 (5.3–7.2) | 7.1 (5.8–8.7) | 7.2 (6.0–8.7) | 6.1 (5.4–6.9) | 6.3 (5.4–7.3) | 8.3 (6.6–10.5) | 9.8 (8.0–12.0) | 11.4 (9.1–14.2) |
| After vaccination 2 | 11.2 (9.1–13.8) | 10.1 (7.8–13.2) | 18.5 (14.2–24.2) | 8.8 (7.2–10.8) | 22.3 (17.3–28.7) | 18.3 (13.7–24.4) | 41.5 (34.4–50.0) | 31.0 (25.6–39.2) |
| Increase in titer,[a] % of subjects | | | | | | | | |
| After vaccination 1 | 3 | 4 | 3 | 0 | 2 | 5 | 11 | 20 |
| After vaccination 2 | 7 | 13 | 24 | 9 | 31 | 34 | 58 | 51 |

**Note.** HA and AlOH levels are per 0.5-mL does of vaccine, unless otherwise indicated.

[a] Per 0.25-mL does of vaccine.

[b] Defined as achievement of a ≥4 fold increase or an increase from <10 to ≥40.