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Randomized Comparative Study of the Serum Antihemagglutinin and Antineuraminidase Antibody Responses to Six Licensed Trivalent Influenza Vaccines

Robert B. Couch[1], **Robert L. Atmar**[1], **Wendy A Keitel**[1], **John Quarles**[2], **Janet Wells**[1], **Nancy Arden**[2], and **Diane Niño**[1]

[1]Baylor College of Medicine, Houston, TX

[2]College of Medicine, Texas A&M Health Science Center, College Station, TX

Abstract

Background—Serum antibody to the hemagglutinin (HA) surface protein of influenza virus induced by influenza vaccinations is a correlate of protection against influenza. The neuraminidase (NA) protein is also on the surface of the virus; antibody to it has been shown to impair virus release from infected cells and to reduce the intensity of influenza infections in animal models and in humans challenged with infectious virus. Recently we have shown that NA inhibiting antibody can independently contribute to immunity to naturally-occurring influenza immunity in the presence of antibody to the HA.

Purpose—The present study was conducted to evaluate induction of antibody to the NA and the HA by commercially available influenza vaccines.

Corresponding Author: Robert B. Couch, M.D., Departments of Molecular Virology & Microbiology and Medicine, Baylor College of Medicine, One Baylor Plaza, MS: BCM280, Houston, TX 77030, 713-798-4474 o, 713-798-8344 f, rcouch@bcm.edu.
Co-Authors:
Robert Atmar, Departments of Medicine and Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-6849 o, 713-798-6802 f, ratmar@bcm.edu
Wendy A. Keitel, Departments of Molecular Virology & Microbiology and Medicine, Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-6849 o, 713-798-6802 f, wkeitel@bcm.edu
John M. Quarles, Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114, 979-845-1358 o, 979-845-3479 f, quarles@medicine.tamhsc.edu
Janet Wells, Department of Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-5420 o, 713-798-6802 f, jwells@bcm.edu
Nancy Arden, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114, 979-845-1358 o, 979-845-3479 f, nancy.arden@prodigy.net
Diane Niño, Department of Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798- 4983 o, 713-798-6802 f, dnino@bcm.edu

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CONFLICTS OF INTEREST

Robert B. Couch, no conflict
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Methods—Healthy young adults were vaccinated with one of five commercially available trivalent inactivated vaccines or live influenza vaccine. Frequencies of serum antibody and fold geometric mean titer (GMT) increases four weeks later were measured to each of the three vaccine viruses (A/H1N1, A/H3N2, B) in hemagglutination-inhibition (HAI) and neutralization (neut) assays. Frequency and fold GMT increase in neuraminidase-inhibition (NI) antibody titers were measured to the influenza A viruses (A/H1N1, A/H3N2).

Results—No significant reactogenicity occurred among the vaccinated subjects. The Fluvirin inactivated vaccine induced more anti-HA antibody responses and a higher fold GMT increase than the other inactivated vaccines but there were no major differences in response frequencies or fold GMT increase among the inactivated vaccines. Both the frequency of antibody increase and fold GMT increase were significantly lower for live vaccine than for any inactivated vaccine in HAI and neut assays for all three vaccine viruses. Afluria inactivated vaccine induced more N1 antibody and Fluarix induced more N2 antibody than the other vaccines but all inactivated vaccines induced serum NI antibody. The live vaccine failed to elicit any NI responses for the N2 NA of A/H3N2 virus and frequencies were low for the N1 of A/H1N1 virus.

Conclusions—Trivalent inactivated influenza vaccines with similar HA dosage induce similar serum anti-HA antibody responses in healthy adults. Current inactivated vaccines all induce serum anti-NA antibody to the N1 and N2 NA proteins but some are better than others for N1 or N2. The live vaccine, Flumist, was a poor inducer of either anti-HA or anti-NA serum antibody compared to inactivated vaccine in the healthy adults. In view of the capacity for contributing to immunity to influenza in humans, developing guidelines for NA content and induction of NA antibody is desirable.

Keywords

Influenza; Vaccination; Antibody; Hemagglutinin; Neuraminidase; Randomized

INTRODUCTION

Influenza is a common acute respiratory disease that occurs annually in human populations. Use of influenza vaccines is the primary means for preventing influenza and vaccines are being increasingly used in populations of all ages. Current licensed trivalent inactivated vaccines (TIVs) are effective for preventing influenza but are less effective than desirable, particularly among the elderly[1,2]. Improvement in vaccines to increase the protection they convey is needed.

The current dosage standard for TIVs is the amount of hemagglutinin (HA) surface protein in the vaccine; serum antibody responses to the HA in hemagglutination-inhibition (HAI) tests are used to define immunogenicity [1,2]. Current TIVs contain 15 μg of the HA of each component; the trivalent live attenuated vaccine (LAIV) contains about $10^{7.0}$ $TCID_{50}$ of each component.

The neuraminidase (NA) surface protein was shown years ago to facilitate virus release from infected cells and its inhibition to impair release and spread of infection [3,4]. That principle was demonstrated in humans where it was shown that selective vaccine induction of NA antibody before infection was followed by a reducing frequency and magnitude of infection and of occurrence and severity of illness among persons when experimentally challenged with influenza virus [5]. Recently, we have shown that serum neuraminidase-inhibition (NI) antibody is an independent predictor of immunity to naturally-occurring influenza in the presence of HAI antibody [manuscript in review]. It is important that the NA protein be present in sufficient quantity to ensure an adequate NA antibody response in vaccinated subjects. The present study used commercially available trivalent influenza vaccines from

six manufacturers for vaccinations of healthy young adults to compare the immune responses to both the HA and NA antigens.

MATERIALS AND METHODS

Subjects

Two hundred two persons were screened for good health and availability; 180 were enrolled in the study. Exclusions were for chronic illnesses, hypertension, new or disallowed medication, recent vaccination, reported allergy to influenza vaccine component, and presence of an unstable illness. Vaccinated subjects were healthy adults between the ages of 18 and 40 years (Table 1). The protocol and consent procedures were reviewed and approved by the Baylor College of Medicine and Texas A&M University Institutional Review Boards for protection of human subjects before commencing the study. The study was conducted in a clinic setting and all subjects gave written informed consent before any procedures were performed.

Vaccines

Six commercially available 2008–2009 TIVs were purchased for the study. Four TIV vaccines were inactivated split-virus products and one was purified subunits; one vaccine was LAIV. The TIVs were: Fluogen, lot U2750aa; Fluarix, lot aflua 401ba; Flulaval, lot aflua166aa; Fluvirin, lot 89980, Afluria, lot 04749111a. The live vaccine was Flumist, lot 500589p. Each TIV contained 15 μg of the HA of each virus. For an O.D. of 2.0, total NA enzyme activities for the TIVs in assays as described were 1:14,000 to 1:50,000 but the contribution to the total by each virus is not known; NA activity for Flumist was 1:8 [6].

Clinical Procedures

The clinical component of the study was conducted between March 25 and April 29, 2009, in the Texas A&M University Health Center at College Station, Texas. After obtaining written consent, a medical history and oral temperature were obtained and a targeted physical examination performed as indicated. Enrolled subjects had a blood specimen obtained and vaccination given. Subjects were randomized in a blinded fashion to receive one of the six influenza vaccines using a computer-generated code in which vaccine allocation occurred in blocks of six. Vaccinations were given by an unblinded vaccinator to a total of 180 subjects (30 in each of six groups). Subjects were blinded to inactivated vaccine received but not those given live vaccine. All other clinical personnel and laboratory technologists were blinded as to the vaccine each person received. All TIV vaccinations were 0.5 ml given intramuscularly using a one inch needle; the LAIV was given intranasally by spray syringe as directed by the manufacturer. Blood for antibody assays was collected again four weeks later on all 180 subjects.

Evaluations

Reactogenicity. Subjects were observed for immediate reactions to vaccination for 20 minutes and provided with a seven day diary for recording oral temperatures and symptoms that was reviewed later with the subject.

Immunogenicity

Serum specimens from pre- and postvaccination bloods were used for antibody assays. Frequency of increase in antibody titer and geometric mean titers (GMT) were compared. Serum antibody titers were determined in hemagglutination-inhibition (HAI) and neutralization tests (neut) as previously described [7,8]. Assay standardizations indicated that a 4-fold increase in titer was significant for both the HAI and neut tests. Serum NI

antibody titers were performed for the influenza A (H1N1) and A (H3N2) vaccine components as previously described [6]. Because of an inability to consistently obtain adequate NA activity to perform NI assays with reagents as prepared in reference 6, we developed an alternative source for NA antigens for NI assays. The NA antigens used for the N1 and N2 proteins were virus-like particles (VLPs); the VLP for N1 assays contained the N1 protein only and was prepared as described [9]. The N2 VLP contained the N2 protein and an irrelevant HA (H5) that was prepared as described [10,11]. Limitation of funds prevented developing an NA reagent and evaluating responses to influenza B. Assay standardizations indicated a two-fold increase in NI titer was significant.

Statistics

Since there are no data permitting a sample size calculation for comparing NI antibody titers and all inactivated vaccines should induce about the same anti-HA antibody response, each of the six vaccines was given to 30 subjects, a number generally suitable for comparisons of anti-HA antibody titers.

Antibody response frequencies were compared in chi square and Fisher exact tests. GMTs and fold increase in GMT were compared in Anova tests with Duncan's post-hoc analysis used for identifying differences between vaccines. Correlations of antibody titers were evaluated in Spearman's rank tests and influence of preexisting antibody in multivariant logistic regression tests. When multiple comparisons were performed, the Holm-Bonferroni method was used to adjust significance levels.

RESULTS

Reactogenicity

No significant reactogenicity occurred for any of the vaccines. Mild and occasionally moderate pain and tenderness at the vaccine site were commonly reported by those given TIV for one to three days after vaccination. One subject reported a moderate headache each day that was called severe on day four; the subject also reported severe malaise on day four after vaccination. One subject reported severe induration only at the vaccination site on days one and two. All other solicited symptoms or local findings were mild and uncommon. Unsolicited reports in the four weeks after vaccination were an acute respiratory illness by four subjects; two were influenza-like, one was a common cold and one was streptococcal pharyngitis. No differences in frequency of reports for the different TIVs were noted. The LAIV reaction reports included frequent reports of mild rhinorrhea. No severe adverse events occurred.

Immunogenicity

Anti-HA Antibody Responses—Serum titers in HAI and neut tests for the H1N1 and H3N2 viruses correlated prevaccination and postvaccination (Spearman's rank test, r 0.704–0.874, $p < .001$ for each. There were significant differences in seroresponse frequencies, final GMT and fold increase in GMT responses for each of the viruses in both HAI and neut tests (Tables 2, 3, 4) (chi square for seroresponse frequencies, $p < .001$ for both tests for the A/H1N1 and A/H3N2 viruses; for B, $p = .002$ for neut and .024 for HAI). In Fisher exact tests, the LAIV seroresponse frequencies were significantly lower for all three vaccine viruses (A/H1N1, A/H3N2 and B) in both assays compared to the inactivated groups combined ($p < .001$ for each virus). Fluvirin had a higher seroresponse frequency in neut for the A/H1N1 and in HAI for A/H3N2 virus ($p \leq .001$ and .01, respectively). There were no differences among the TIVs for influenza B.

There were no differences among the vaccines in the preimmunization GMTs but there were differences in postimmunization GMTs and fold increase in GMT among the vaccines for each virus (Anova, p <.001 for each assay and virus). The postvaccination means were all significantly higher than prevaccination for all vaccines except Flumist for H1 HAI titers and H3 and B neut titers (paired t test, p ≤ .008 for each). Post-hoc comparisons indicated the LAIV post immunization GMT and fold increase were significantly lower than for the TIVs for each virus and assay (Duncan's post-hoc analysis). Among the TIVs, the postimmunization GMT values for Fluvirin were higher in HAI than for Flulaval for A/H1N1; the fold increase in GMT was higher for Fluvirin than for Fluarix in neut for A/H3N2.

Anti-NA Antibody Responses—Serum NI titers for the N1 and N2 antigens correlated with the prevaccination and postvaccination HAI and neut titers (Spearman's rank test, r 0.356–0.437, p<.001 for each for pre titers; for post titers, r 0.200–0.356 for N1 and 0.464–0.473 for N2 with p = .007 for HAI and .013 for neut for H1N1 and p <.001 for HAI and neut for H3N2). The NI frequencies and GMT for the A/H1N1 and A/H3N2 viruses in each vaccine are shown in Tables 5 and 6. There were differences between vaccines in frequency of seroresponses (chi square, p=.017 for A/H1N1 and <.001 for A/H3N2); there were no NI responses for the LAIV-T for the A/H3N2 virus (Fisher's Exact Test, p <.001). For Afluria, the 57% increase for the NI of A/H1N1 was significantly higher as was the 73% to the N2 of A/H3N2 for Fluarix than were increases for the other vaccines (Fisher's Exact, p=.005 and .002, respectively).

The preimmunization GMTs for each virus were not different; differences in postimmunization GMTs for the A/H1N1 virus were not significant but were for the A/H3N2 virus (Anova, p < .001). The mean fold increase in titers was different for each virus (A/H1N1, p=.019; A/H3N2, p < .001). All postvaccination titer means were significantly higher than prevaccination for all vaccine groups except Flumist for N2 (paired t test, p ≤ .008 for each). The postimmunization GMT and fold increase for the N2 of A/H3N2 was significantly lower for LAIV than for the other vaccines (Duncan's post-hoc analysis). The fold increase in GMT was higher for Afluria for A/H1N1 than for Flumist, Fluarix and Flulaval and for Fluarix than for Flumist, Fluzone and Afluria for A/H3N2 (Duncan's post-hoc analysis).

Responses in Relation to Prevaccination Antibody—In multivariate analyses the higher the prevaccination anti-HA antibody titer the lower the percent with an increase and fold increase in both H1N1 and H3N2 HAI and neut tests (p <.001 for each virus and assay). Similarly, the higher the prevaccination anti-NA antibody titer, the lower the percent with an increase and fold increase in titer for both the N1 and N2 antigens (p <.001 for each antigen). Higher anti-HA antibody titers initially did not relate to increases in NA antibody titers of either the N1 or N2 antigen and higher anti-NA titers initially did not relate to increases in either anti-HA antibody test of either H1N1 or H3N2 virus (p >.10 for each). The higher the prevaccination titer of anti-HA and anti-NA antibody, the higher the postvaccination titer (p <.001 for each test); for N2, the higher the pre-N2 titer, the higher the postvaccination titer of both H3 and N2 (p <.001 for each).

DISCUSSION

The immune response comparisons in this study detected some differences in responses to the HA and NA for the different vaccines. In serum HAI and neut tests, responses to the LAIV were lower for each of the three viruses in each of the antibody tests than were responses to any of the TIVs. Fluvirin vaccine tended to be somewhat more immunogenic for anti-HA antibody for the two influenza A components but there were no differences

among the other inactivated vaccines. There were no differences for influenza B responses among the different TIVs. In general, the TIVs induced similar responses in HAI and neut tests; only the LAIV was noticeably lower.

Annual vaccine recommendations by WHO are for strains to be included in the vaccines for the coming year with dosage recommendations for inactivated vaccines of 15 μg of the HA of each component [1]. Although manufacturing processes are different for each commercially available vaccine, the assumption is that each will induce similar anti-HA immune responses. It is reassuring to see data indicating the assumption is correct for current vaccines for healthy adults. However, differences in responses by vaccine and population are possible. Probably the most extensive comparisons of different inactivated vaccines conducted were those for A/New Jersey/76 (H1N1) and A/USSR/77 (H1N1) vaccines in 1976 and 1978 [12,13]. Vaccines of whole virus and split product, vaccines from different manufacturers, vaccines containing different HA dosages and results in different age groups were compared. Although results varied, in general, whole virus vaccines were more immunogenic in unprimed persons, and increasing dosage increased responses; some differences between manufacturers' preparations were noted. In a recent comparison of two inactivated preparations in children, responses were similar for older children but one of the commercially-available preparations was inferior to the other for infants 6 to 36 months of age [14]. A focus on serum anti-HA antibody is appropriate as the correlation of serum anti-HA antibody titer with immunity to influenza infections and illness has been repeatedly demonstrated since first described in the 1930s and 1940s [15–18].

Antibody to the NA of each of the influenza A subtypes was also evaluated. There were significant differences between vaccine responses for each of the type A viruses. The anti-NA antibody responses in both frequency and GMT were lower for live vaccine than for any of the TIVs. Afluria vaccine responses in frequency and GMT were greater for the N1 antigen of A/H1N1 virus while responses to Fluarix vaccine were greater for the N2 of the A/H3N2 virus. Nevertheless, all of the inactivated vaccines induced NI antibody indicating immunogenic NA antigen for both N1 and N2 was present in each vaccine. Thus, the different manufacturing processes preserved conformational NA protein of each type A protein.

There have been a number of reports over the past several decades of NA antibody responses to vaccine and of a potential role for NA antibody in human influenza [5,6,19–30]. Serum antibody to the NA was shown years ago to correlate with immunity to influenza virus infection and illness in humans [5,20,30]. Past reports have identified several needs to ensure NA antibody responses from vaccinations. Kendal, et al. identified and emphasized the problem of variable NA dosage and antigenic stability in the A/New Jersey/76(H1N1) and A/USSR/77(H1N1) coordinated studies of inactivated vaccines [21,22]. Notable in those assessments was the complete absence of NA activity in the A/New Jersey monovalent vaccines. Considerable variability in antibody response was detected with response frequencies less than 50% in his assay. Kendal, et al. suggested that anti-HA antibody might interfere with anti-NA responses; a suggestion later confirmed by Johansson and Kilbourne [31]. Other studies have reported low NI response frequencies (<50%) to inactivated vaccine in young adults while higher frequencies have been reported in children [23,24,26–28]. Interpreting these varied responses is difficult in the absence of knowledge of NA dosage and the variability of assay methods used.

The limited data available of vaccine NA dosages have indicated varied enzyme activity of inactivated vaccines with NA protein in 15 μg HA vaccines of two to 10 μg [21,22,25,32]. In a dose response study with an NA vaccine, serum antibody responses progressively increased with increasing NA dosage [25]. Also, in an earlier study, we measured serum

anti-NA antibody responses among elderly persons given a standard TIV containing 15 μg of the HA of each component and a group given 60 μg of the HA of each [6]. Both the HA concentration and enzyme activity were increased for the higher dosage vaccine and both serum anti-HA and anti-NA antibody responses were increased. Antigen dosage is an important consideration for induction of anti-NA antibody responses.

An additional concern for interpreting antibody response to influenza vaccines in humans is the assay used for measuring those responses. Several assays for NA protein and NA antibody have been used and have likely contributed to the varied immunogenicity data. The initial assays used influenza viruses with an irrelevant HA as antigen and a thiobarbituric acid detection assay for detecting release of neuraminic acid from fetuin and its inhibition by antibody [33]. Standards for increases in antibody titer have usually been for a three- or four-fold increase. More recently, lectin-based assays have been used for detecting NA activity with a fetuin substrate [34]. We used a lectin-based assay and a split-virus antigen for antibody detection in a vaccine study in the elderly and showed in standardized assays that within test variation were sufficiently low to permit a two-fold increase as being significant [6]. However, we were unable to reproducibly develop split-virus pools of some other strains to use that method as a routine antigen source. As indicated in methods, we adopted VLPs expressing NA as antigens for assays in this report. Standardization studies indicated a two-fold difference between sera had high specificity in these assays. Using two-fold as significance, we found moderate response frequencies in a young adult population for the N1 and N2 proteins of influenza A viruses as presented in this report. The assay identified subjects with a titer range of <1:8 to >1:1024. A comparison of the standard thiobarbituric acid assay to a lectin based assay using a recombinant NA as antigen showed greater sensitivity for the lectin based assay [29]. Assay differences could account for the greater frequency of antibody responses to the NA of A/Brisbane/59/07 in young adults in our study that were induced by 2008–2009 TIVs than were reported earlier for a 2008–2009 TIV [28].

The apparent deficiency for inducing HA and NA serum antibody by LAIV may be because of high pre-existing immunity of subjects to the viruses in the vaccine that served to restrict the level of virus replication. Studies in this age group with other influenza A and B viruses have shown a low level of virus shedding [35–37]; however, virus replication among young children has been greater than for adults [38]. While the definition of population groups where LAIV or TIV is the preferred vaccine is still evolving, comparisons of efficacy have suggested LAIV induces greater protection in relatively unprimed populations, particularly children [39–47]. In a study of LAIV, presence of some preexisting serum antibody at the time of LAIV administration did not reduce the efficacy compared to TIV for children less than five years old [48]. Other data suggest continued high efficacy for LAIV among adolescents and young military recruits [40,45,46,48]. Thus far, however, TIV appears to be more efficacious in older adults and is proposed to be because of a reduced level of replication and serum anti-HA antibody responses for LAIV[42,44–46]. This belief is compatible with studies of the correlates to immunity induced by LAIV vaccinations in children which indicated that serum HAI antibody (and nasal wash IgA antibody), correlated with protection [49].

The primary purpose of the present study was to evaluate induction of NI antibody by existing commercial vaccines. It is reassuring that all TIVs contained NA antigen that induced NI antibody responses. The LAIV responses were lower for the N1 and N2 than those for inactivated vaccines; one of the TIVs was a better inducer of NI antibody than the others for N1 and a different vaccine was better for N2. Since inactivated vaccines need improvement and NI antibody has been shown recently to be an independent predictor of

immunity to influenza in humans, developing guidelines for induction of anti-NA antibody by licensed vaccines is appropriate.

In summary, current split and subunit TIVs induce similar serum anti-HA antibody responses in healthy adults; moreover, each contains conformationally intact NA antigen that induces serum NI antibody. A limitation of the NA evaluations was an inability to evaluate NA antibody responses for influenza B. Nevertheless, since currently available data indicate a capacity for a significant contribution of serum NI antibody in immunity to influenza in humans, efforts to develop guidelines for ensuring induction of the antibody by licensed vaccines seems appropriate.

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Highlights

- Minimal reactogenicity for 5 inactivated vaccines and a live vaccine
- Similar serum anti-hemagglutinin antibody responses to 5 inactivated vaccines
- All 5 inactivated vaccines induced serum anti-neuraminidase antibody

Table 1

Demography of the Study Population

Total subjects	180
Gender	
Male	90 (50%)
Female	90 (50%)
Race and Ethnic Group	
White	162
Hispanic	32
Non-Hispanic	130
Black	7
Asian	7
American Indian	1
Multiracial	3
Age (Years)	
Mean	22.3
Median	21.3
Range	18 to 40

Table 2

Frequency of Serum Antihemagglutinin Antibody Responses of Healthy Adults to 2008–2009 Influenza Vaccines[1]

	A/Brisbane/59/07 (H1)		A/Brisbane/10/07 (H3)		B/Florida/4/06	
Vaccine	**HAI[2]**	**Neut[2]**	**HAI[2]**	**Neut[2]**	**HAI[2]**	**Neut[2]**
Afluria	14 (47)	20 (67)	17 (57)	22 (73)	11 (37)	14 (47)
Fluarix	19 (63)	21 (70)	18 (60)	19 (63)	9 (30)	16 (53)
Flulaval	13 (43)	20 (67)	20 (67)	23 (77)	6 (20)	18 (60)
Fluzone	14 (47)	19 (63)	15 (50)	19 (63)	10 (33)	18 (60)
Fluvirin	17 (57)	27 (90)	22 (73)	23 (77)	11 (37)	16 (53)
Flumist	2 (7)	4 (13)	1 (3)	1 (3)	1 (3)	4 (13)

[1]Number (%) with four-fold increase in titer; 30 per group

[2]Hemagglutinination-inhibiton (HAI) and neutralization (neut) antibody

Comparisons: Chi square for difference among all vaccines, H1 and H3 p <.001 for HAI and neut, p=.024 for B HAI and p=.002 for neut. Fisher exact, Flumist less than others, p ≤ .001 for all viruses and assays. Fluvirin greater for H3 in HAI (p=.01) and for H1 in neut (p <.001)

Table 3

Geometric Mean Serum Hemagglutination-inhibition Antibody Titer[1] and Mean Fold Increase of Healthy Adults Given 2008–2009 Influenza Vaccines[2]

Vaccine	A/Brisbane/59/07 (H1)			A/Brisbane/10/07 (H3)			B/Florida/4/06		
	Pre	Post	Fold Increase	Pre	Post	Fold Increase	Pre	Post	Fold Increase
Afluria	4.30	6.47	2.17	5.22	7.78	2.57	4.22	6.10	1.88
Fluarix	3.85	6.48	2.63	5.40	8.02	2.62	4.52	6.10	1.58
Flulaval	4.02	6.20	2.18	5.57	8.68	2.72	4.52	6.33	1.82
Fluzone	4.65	6.65	2.00	5.52	7.93	2.42	4.42	6.27	1.85
Fluvirin	4.07	6.70	2.63	5.00	8.25	3.25	4.38	6.20	1.82
Flumist	4.32	4.68	0.37	5.38	5.73	0.35	4.33	4.75	0.42

[1] GMT – $\log_2$

[2] N=30 in each group

Comparisons: Anova for differences among vaccines, pre NS for all, post and fold increase, $p < .001$ for each. Duncan's post-hoc for differences among vaccines, Flumist lower for all viruses and assays post and fold increase, Fluvirin post greater than Flulaval for H1.

Table 4

Geometric Mean Serum Neutralizing Antibody Titer[1] and Mean Fold Increase of Healthy Adults Given 2008–2009 Influenza Vaccines[2]

	A/Brisbane/59/07 (H1)			A/Brisbane/10/07 (H3)			B/Florida/4/06		
Vaccine	**Pre**	**Post**	**Fold Increase**	**Pre**	**Post**	**Fold Increase**	**Pre**	**Post**	**Fold Increase**
Afluria	4.80	8.83	4.03	4.03	7.53	3.50	6.98	9.65	2.67
Fluarix	3.87	8.43	4.57	4.28	7.37	3.08	7.47	10.12	2.65
Flulaval	3.85	7.72	3.87	4.62	8.60	3.98	7.65	10.43	2.78
Fluzone	5.13	8.75	3.62	4.45	8.00	3.55	7.25	10.23	2.98
Fluvirin	4.42	9.50	5.08	3.68	8.00	4.32	7.50	10.47	2.97
Flumist	4.73	5.60	0.87	4.05	4.35	0.30	7.50	7.90	0.40

[1] GMT – $\log_2$

[2] N=30 in each group

Comparisons: Anova for differences among vaccines, pre NS for all, post and fold increase, $p < .001$ for each. Duncan's post-hoc for differences among vaccines, Flumist lower for all viruses and assays post and fold increase. Fluvirin fold increase higher than Fluarix for H3.

Table 5

Frequency of Serum Neuraminidase-Inhibition Antibody Responses of Healthy Adults to 2008–2009 Influenza Vaccines[1]

Vaccine	A/Brisbane/59/07 (N1)	A/Brisbane/10/07 (N2)
Afluria	17 (57)	14 (47)
Fluarix	7 (23)	22 (73)
Flulaval	8 (27)	18 (60)
Fluzone	12 (40)	14 (47)
Fluvirin	11 (37)	17 (57)
Flumist	5 (17)	0 (0)

[1] Number (%) with two-fold increase in titer; 30 per group

Comparisons: Chi square for differences among vaccines, N1 p=.017, N2 p <.001. Fisher exact, Flumist less than others, p < .001 for N2. Afluria greater for N1 (p=.005) and Fluarix greater for N2 (p=.002).

Table 6

Geometric Mean Serum Neuraminidase-Inhibition Antibody Titer and Mean Fold Increase of Healthy Adults to 2008–2009 Influenza Vaccines[1]

Vaccine	A/Brisbane/59/07 (N1)			A/Brisbane/10/07 (N2)		
	Pre	Post	Fold Increase	Pre	Post	Fold Increase
Afluria	7.52	8.78	1.26	7.76	8.90	1.14
Fluarix	7.35	8.02	0.66	7.31	9.35	2.04
Flulaval	7.45	8.06	0.61	7.36	9.07	1.71
Fluzone	7.92	8.95	1.02	7.89	9.14	1.25
Fluvirin	7.77	8.80	1.02	7.26	8.91	1.65
Flumist	7.66	8.13	0.47	7.77	7.94	0.17

[1] $GMT - \log_2$

[2] N=30 in each group

Comparisons: Anova for differences between vaccines, pre = NS for N1 and N2, post p=.051 for N1 and <.001 for N2, fold increase p=.019 for N1 and < .001 for N2. Duncan's post hoc for differences among vaccines; the fold increase for N1 was greater for Afluria than for Flumist, Fluarix and Flulaval. Fluarix was greater than Afluria, Fluzone and Flumist for N2.