Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age (US Flu VE Serologic Study, 2016-17 and 2017-18)

Vaccines	Trivalent Standard Dose Inactivated Influenza Vaccine (Fluvirin, Seqirus)(IIV3) Trivalent High Dose Inactivated Influenza vaccine (Fluzone High-Dose, Sanofi Pasteur)(HD-IIV3) Trivalent Inactivated Influenza Vaccine with adjuvant (Fluad, Seqirus)(aIIV3) Quadrivalent Recombinant Influenza Vaccine (FluBlok, Protein Sciences)
Sponsor	US Centers for Disease Control and Prevention (CDC) 1600 Clifton Road Atlanta, GA 30329
CDC Project Officers	Brendan Flannery, PhD Alicia Fry, MD
Marshfield Clinic Research Institute Investigators and Key Personnel	Huong McLean, PhD Edward Belongia, MD Jennifer Meece, PhD Jennifer King, MPH
Version and Date	Version 3.0 – July 2017

PROTOCOL CHANGE HISTORY

Date/Version	Change
Dec 2016	Change to illness lockout period (page 10, 19).
July 2017; Version 3.0	Add new influenza vaccine for Year 2; Add 30 new participants for Year 2

.

Table of Contents

GLOSSARY OF ABBREVIATIONS

Abbreviation or Term	Definition
ADCC	Antibody dependent cellular cytotoxicity
aIIV3	Trivalent inactivated influenza vaccine with adjuvant
CDC	US Centers for Disease Control and Prevention
CI	Confidence interval
GMT	Geometric mean titers
HD-IIV3	High-dose trivalent inactivated influenza vaccine
HI	Hemagglutination inhibition
IM	Intramuscular
IIV3	Standard dose trivalent inactivated influenza vaccine
MFR	Mean fold rise
MN	Microneutralization
NAI	Neuraminidase inhibition
RIV4	Quadrivalent recombinant influenza vaccine
SD	Standard deviation
SPR	Seroprotection rate
SRR	Seroresponse rate

CLINICAL PROTOCOL SYNOPSIS

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

Regimen and Dosing: Subjects will receive a 0.5 mL intramuscular (IM) injection of one of three licensed 2016-17 influenza vaccines at study visit 1 and either 2017-18 Fluzone High-Dose (HD-IIV3), Fluad (aIIV3) or FluBlok (RIV3) at study visit 4 (approximately 1 year after enrollment).

Study Rationale:

Influenza vaccination is recommended annually for all persons aged ≥6 months. Several newer vaccines have been developed and are recommended for persons aged ≥65 years, including vaccines with increased amounts of antigen (high dose) or adjuvant, to increase immune response. The recombinant influenza vaccine has also shown high effectiveness in older adults. Immunologic response to influenza vaccination is dependent on age and pre-existing antibody levels from prior exposure either to vaccination or disease. Recent observational studies of influenza vaccine effectiveness have suggested that prior season vaccination status may be an important factor in vaccine protection, but studies have been inconsistent. Limited data suggest that repeat high-dose vaccination does not interfere with immune response while there are no data on immune response to repeat vaccination with adjuvanted vaccines.

Given the universal recommendation for annual vaccination for all adults, increased risk of complications from influenza infection in older adults, and the availability of vaccines specifically formulated to increase immune response to vaccination for persons aged ≥65 years, a better understanding of the humoral immune response to repeat influenza vaccination and vaccine type in this population is needed. There is currently no preferential recommendation for a specific vaccine for the population aged ≥65 years. Understanding immune responses to these newer vaccines compared to standard dose vaccines upon vaccination and revaccination will help to guide vaccine policy for this age group.

Study Objectives:

Primary:

- Compare immune responses to vaccine and drifted viruses as measured by hemagglutination inhibition (HI) among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines: high-dose trivalent inactivated influenza vaccine (HD-IIV3) or trivalent inactivated influenza vaccine with adjuvant (aIIV3) approximately 28 days, 6 months, and 1 year after receipt of the 2016-17 vaccine
- Compare immune responses to vaccine and drifted viruses as measured by HI among subjects after repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

Secondary:

- Compare immune responses as measured by virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines: HD-IIV3 or aIIV3 approximately 28 days, 6 months, and 12 months after receipt of the 2016-17 vaccine.
- Compare immune responses as measured by virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers among subjects after repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 with subjects vaccinated approximately 28 days and 6 months after receipt of the 2017-18 vaccine.
- Compare immune responses as measured by HI by prior receipt of 2015-16 influenza vaccine among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines: HD-IIV3 or aIIV3 approximately 28 days, 6 months, and 1 year after receipt of the 2016-17 vaccine.
- Compare immune responses as measured by HI among persons aged 65-74 years who received standard dose trivalent inactivated influenza vaccine (IIV3) in 2016-17 and vaccinated with one of three licensed influenza vaccines in 2017-18: HD-IIV3, aIIV3, or RIV4 approximately 28 days, and 6 months after receipt of the 2017-18 vaccine.
- Compare immune responses as measured by HI among persons who received IIV3 in 2016-17 and vaccinated with one of two licensed influenza vaccines in 2017-18: HD-IIV3 oraIIV3 with subjects who received repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.
- Conduct influenza surveillance to determine factors associated with vaccine failure (laboratory confirmed influenza) among study participants if it occurs during the surveillance period.

Study Endpoints:

Primary:

- Hemagglutinin inhibition (HI) antibody levels specific for vaccine strains and representative strains of circulating influenza viruses that are antigenically or genetically different. Endpoints based on these data will include geometric mean titers (GMT), mean fold rise (MFR), seroresponse rate (SRR), and seroprotection rate (SPR).
    - Seroresponse rate defined as the proportion of subjects with paired samples with prevaccination HI titer <1:10 and postvaccination (day 28) HI titer ≥1:40 or at least a 4-fold increase in HI from prevaccination HI titer ≥1:10.
    - Seroprotection rate defined as the proportion of subjects with postvaccination HI titer ≥1:40.

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

Secondary:

- Virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers specific for vaccine strains and representative strains of circulating influenza viruses that are antigenically or genetically different. Endpoints based on these data will include geometric mean titers (GMT) and mean fold rise (MFR).
- RT-PCR confirmed influenza

Study Design:

This is an open-label, randomized trial with enrollment of up to 180 adults aged 65-74 years in the first year (2016-17) and an additional 30 adults in the second year (2017-18) for a total of 210 across two years.

Randomization to a vaccine group in the first year (2016-17) will be stratified by receipt of 2015-16 influenza vaccine. The randomization will target enrollment of approximately 25% of subjects in the unvaccinated in 2015-16 stratum. It is anticipated that a percentage of the randomized study subjects may not complete the study; subjects who withdraw or are discontinued may be replaced at the beginning of the 2017-18 influenza season.

Vaccines administered will include a single IM dose of one of three licensed 2016-17 influenza vaccines at study visit 1. At study visit 4, subjects who received HD-IIV3 and aIIV3 at visit 1 will receive the same vaccine type for the 2017-18 influenza season. Subjects who received IIV3will be randomized to receive HD-IIV3, aIIV3 or RIV4. An additional 30 individuals who received IIV3 in the 2016-17 season will be recruited for study visit 4 (the beginning of the 2017-18 influenza season) and randomized to receive HD-IIV3, aIIV3 or RIV4.

	Group	2016-17 Vaccine	2017-18 Vaccine	Total
65-74 years	A	2016-17 HD-IIV3	2017-18 HD-IIV3	60
	B	2016-17 aIIV3	2017-18 aIIV3	60
	C1	2016-17 IIV3	2017-18 HD-IIV3	30
	C2	2016-17 IIV3	2017-18 aIIV3	30
	C3	2016-17 IIV3	2017-18 RIV4	30
		Total		210

For each subject enrolled in the first year, study follow-up will span approximately two years from the first dose. Study follow-up will span approximately 6 months from their enrollment date for study subjects recruited in Fall 2017.

Eligibility Criteria:

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

Inclusion:

Subjects must meet all of the following criteria to be eligible to participate:

1) Age 65-74 years at the time of study enrollment and are ambulatory and live in Marshfield, Wisconsin or surrounding communities.
2) Willing and able to give informed consent prior to study enrollment.
3) Able to comply with study requirements.

Exclusion:

Subjects will be excluded if they fulfill any of the following criteria:

1) Prior receipt of 2016-17 (or 2017-18 for participants recruited in Fall 2017) influenza vaccine.
2) Current participation in another clinical trial.
3) Presence of a contraindication to influenza vaccination.

Study Visit Procedures:

Subjects recruited in Fall 2016 will complete all the following study visits and acute respiratory illness during the 2016-17 and 2017-18 influenza seasons. Subjects recruited in Fall 2017 will complete visits 4 through 6, and surveillance for acute respiratory illness during the 2017-18 influenza season.

Visit 1 – Screening

Adult volunteers aged 65-74 years who have provided written informed consent and are able to comply with study requirements will have the following procedures performed: inclusion and exclusion criteria review; measurement of height and weight; review and recording of concomitant medications. Potential subjects who meet all inclusion criteria and none of the exclusion criteria may be enrolled.

Visit 1 – Vaccination

All subjects who have eligibility confirmed will have blood draw (20 mL) for baseline serologic evaluation (including hemagglutination inhibition and other assays).

Subjects will then be randomized to one of three vaccine group and administered the 2016-17 influenza vaccine using standard procedures for IM injection of the licensed vaccines.

The remaining study visits will be scheduled.

Visit 2 – Follow-Up

Subjects will return to the clinic approximately 28 days (±5 days) following receipt of the 2016-17 influenza vaccine. A blood sample (20 mL) will be obtained for serologic evaluation of influenza vaccine responses. Instructions for illness monitoring will be given to subjects and the next study visit will be confirmed.

Visit 3 – Follow-Up

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

All subjects will return to the clinic approximately 182 days (±10 days) following receipt of the 2016-17 influenza vaccine. A blood sample (20 mL) will be obtained for serologic evaluation of influenza vaccine responses. The study visit will be confirmed.

Visit 4 – Follow-up and Vaccination

Subjects will return approximately 1 year following receipt of the 2016-17 influenza vaccine. A blood sample (20 mL) will be obtained for serologic evaluation (including hemagglutination inhibition and other assays). Subjects who received high-dose inactivated influenza vaccine and inactivated vaccine with adjuvant in 2016-17 will receive the same vaccine type for the 2017-18 season. Subjects who received standard dose inactivated vaccine in 2016-17 will be randomized to receive high-dose vaccine, vaccine with adjuvant, or the recombinant vaccine. The vaccines will be administered using standard procedures for IM injection of the licensed vaccines. The next study visits will be confirmed.

An additional 30 subjects will be added to the study. Adult volunteers aged 65-74 years who have provided written informed consent and are able to comply with study requirements will have the following procedures performed: inclusion and exclusion criteria review; measurement of height and weight; review and recording of concomitant medications. All subjects who have eligibility confirmed will have blood draw (20 mL) for baseline serologic evaluation (including hemagglutination inhibition and other assays). Subjects will then be randomized to receive high-dose vaccine, vaccine with adjuvant, or the recombinant vaccine and administered the 2017-18 influenza vaccine using standard procedures for IM injection of the licensed vaccines. The remaining study visits will be scheduled.

Visit 5 – Follow-Up

Subjects will return to the clinic approximately 28 days (±5 days) following receipt of the 2017-18 influenza vaccine. A blood sample (20 mL) will be obtained for serologic evaluation of influenza vaccine responses. Instructions for illness monitoring will be given to subjects and the next study visit will be confirmed.

Visit 6 – Follow-Up

All subjects will return to the clinic approximately 182 days (±10 days) following receipt of the 2017-18 influenza vaccine. A blood sample (20 mL) will be obtained for serologic evaluation of influenza vaccine responses.

Time and Events Schedule

Visit	1	2	3	4	5	6
Approximate study day	0	28	182	365/0*	393/28*	547/182*
Informed consent	X					
Inclusion/exclusion criteria review	X					

Study Sponsor: CDC

Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age

Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)

Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok

Influenza serologic evaluation	X	X	X	X	X	X
Influenza vaccination	X			X		
Respiratory illness surveillance		11/1/16-4/1/17			11/1/17-4/1/18	

*For participants recruited and enrolled in Fall 2017

Surveillance for Acute Respiratory Illness:

From approximately November 1, 2016 through April 1, 2017 and November 1, 2017 through April 1, 2018, subjects will enter passive and active surveillance for acute respiratory illness. Surveillance will include:

- Instructions to contact the study site with onset of new acute respiratory illness.
- Weekly calls from study staff to solicit respiratory illness

"New onset" will require a period free of symptoms, or with baseline symptoms, and at least 14 days from onset of previous illness to differentiate an episode from any prior illness. When a new acute respiratory illness is reported and confirmed, the subject will be invited to return to the clinic, or study staff will make a home visit to collect a nasal and throat swab within 7 days of illness onset for influenza testing.

Subjects will continue to receive weekly telephone calls for ascertainment of respiratory symptoms. Subsequent weekly calls will document resolution, or return to baseline, of symptoms.

Statistical Methods:

General

Demographic and other baseline characteristics will be summarized by treatment group for all subjects. Continuous variables will be presented by summary statistics (e.g., mean, and standard deviation (SD)) for non-immunogenicity endpoints, and geometric means and their 95% confidence interval for the immunogenicity endpoints. Categorical variables will be presented by frequency distributions; i.e., frequency counts and percentages for the non-immunogenicity endpoints, and the percentages and their 95% confidence intervals (CI) for the immunogenicity endpoints.

Analyses of Primary Objectives

Geometric mean HI antibody titers at each time point will be compared by vaccine group. For the primary aim, adults receiving high dose trivalent inactivated influenza vaccine will be compared with those receiving aIIV3. Linear mixed-effects models will be used to adjust for variables such as age, given the potential for small differences in age and the known difference in GMT by age, baseline antibody titer and prior season vaccination status. The mean fold rise (MFR), defined as the mean of the ratio of post vaccination GMT and pre-vaccination GMT for each subject, between the groups will be computed from mixed-effects model along with its associated 95% CI.

Study Sponsor: CDC
Title: Open-Label, Randomized Study of Immune Response to Licensed Influenza Vaccines in Adults 65-74 Years of Age
Study short name: Open-Label Influenza Vaccine Evaluation (OLIVE)
Vaccines: Fluvirin, Fluzone High-Dose, Fluad, FluBlok
Analyses of Secondary Objectives Analyses of virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers will be similar to HI data described above. If vaccine failures (RT-PCR confirmed influenza infection) occur, we will assess factors associated with failure using logistic regression, including vaccine type. ***Power Considerations*** A sample size of 60 per group will provide over 90% power using a one-sided, two-sample t-test to conclude that one vaccine group is superior as defined by rejecting a null hypothesis of equality if the GMT values differ by approximately 20%.

1 BACKGROUND AND SIGNIFICANCE

Influenza is an important cause of death and serious illness among persons aged ≥65 years in the United States. Recommendations for routine annual influenza vaccination have traditionally targeted persons aged ≥65 years because most influenza deaths occur in this age group.[1-7] However, despite high vaccination coverage in this population, high rates of influenza-associated hospitalizations continue to occur. One explanation may be poor response to traditional, standard dose inactivated influenza vaccines. Several newer vaccines have been developed and are recommended for persons aged ≥65 years, including vaccines with increased amounts of antigen (high dose) or adjuvant, to increase immune response. One advantage of adjuvanted vaccines may be cross-protection against antigenically drifted influenza viruses,[8] while cross-protection provided by high-dose vaccine is unknown. Additionally, recombinant influenza vaccine, licensed for all adults aged ≥18 years, has been shown to provide better protection than standard dose influenza vaccine against influenza A among adults aged ≥50 years during a season with an antigenic mismatch between circulating and vaccine influenza strains (relative efficacy: 36; 95% CI 14 to 53){Dunkle, 2017 #2603}. There is currently no preferential recommendation for a specific vaccine for the population aged ≥65 years, and all influenza vaccines that will be used in this study are licensed and recommended for use in this population. Understanding immune responses to these newer vaccines compared to standard dose vaccines upon vaccination and revaccination will help to guide vaccine policy for this age group.

The immune response to influenza vaccine in older adults, particularly those with chronic disease, is less well studied than that of younger, healthier adults and children. Immunosenescence likely increases the risk of disease in older adults while at the same time, decreases the desired response to vaccination.[9] Moreover, while hemagglutinin inhibition (HAI) titers have typically been used to evaluate immune response to vaccination, with a 4-fold or greater increase in titers associated with a 50% reduction in infection,[10] very few studies have applied newer, state-of-the-art technologies to also measure cell-mediated immune response to vaccination. An improved understanding of the magnitude and breadth of the full immune response to vaccination is needed.

Immunologic response to influenza vaccination is dependent on age and pre-existing antibody levels from prior exposure either to vaccination or disease. Recent observational studies of influenza vaccine effectiveness have suggested that prior season vaccination status may be an important factor in vaccine protection. Serologic studies examining antibody response to vaccination among persons with and without prior history of vaccination have been inconsistent. Some serologic studies have found stronger responses to vaccination in previously unvaccinated subjects compared to those who were revaccinated,[11-13] some found stronger responses in those revaccinated,[14] and some found no differences in responses.[15,16] Limited data suggest that repeat high-dose vaccination does not interfere with immune response while there are no data on immune response to repeat vaccination with adjuvanted vaccines.[17]

Given the universal recommendation for annual vaccination for all adults and the availability of vaccines specifically formulated to increase immune response to vaccination for persons aged ≥65 years, a better understanding of the humoral immune response to repeat influenza vaccination and vaccine type in this population is needed. We propose to conduct a prospective serologic study with assignment of vaccine type among persons aged 65-74 years to better understand the impact on immune response of vaccination and repeat vaccination with differing types of vaccines. The findings could have implications on the current vaccine policy if there is evidence of differing immune response given the type of vaccine received or the order in which specific types are received.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- Compare immune responses to vaccine and drifted viruses as measured by hemagglutination inhibition (HI) among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines recommended for this age group specifically formulated to increase immune response to vaccination: HD-IIV3 or aIIV3 approximately 28 days, 6 months, and 12 months after receipt of the 2016-17 vaccine.
- Compare immune responses to vaccine and drifted viruses as measured by HI among subjects after repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.

2.2 Secondary Objectives

- Compare immune responses as measured by virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines: HD-IIV3 or aIIV3 approximately 28 days, 6 months, and 12 months after receipt of the 2016-17 vaccine.
- Compare immune responses as measured by virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers among subjects after repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 with subjects vaccinated with IIV3 in 2016-17 and either HD-IIV3 and aIIV3 in 2017-18 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.
- Compare immune responses as measured by HI by prior receipt of 2015-16 influenza vaccine among persons aged 65-74 years vaccinated with one of two licensed influenza vaccines: HD-IIV3 or aIIV3 approximately 28 days, 6 months, and 1 year after receipt of the 2016-17 vaccine.
- Compare immune responses as measured by HI among persons aged 65-74 years who received IIV3 in 2016-17 and vaccinated with one of three licensed influenza vaccines in

2017-18: HD-IIV3, aIIV3 or RIV4 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.

- Compare immune responses as measured by HI among persons who received IIV3 in 2016-17 and vaccinated with one of two licensed influenza vaccines in 2017-18: HD-IIV3 or aIIV3 with subjects who received repeat influenza vaccination (2016-17 and 2017-18) with HD-IIV3 and aIIV3 approximately 28 days and 6 months after receipt of the 2017-18 vaccine.
- Conduct influenza surveillance to determine factors associated with vaccine failure (laboratory confirmed influenza) among study participants if it occurs during the surveillance period.

3 STUDY DESIGN

3.1 Design

This is a randomized, open-label serologic study with enrollment of up to 180 adults aged 65-74 years in the first year (2016-17) and an additional 30 adults in the second year (2017-18) for a total of 210 across two years, at Marshfield Clinic Research Institute (MCRI). Subjects will be recruited from community-dwelling individuals who receive their inpatient and outpatient care from Marshfield Clinic facilities.

Randomization to one of three licensed influenza vaccine groups in the first year (2016-17) will be stratified by prior receipt of the 2015-16 influenza vaccine. The randomization algorithm will target enrollment of approximately 25% of subjects in the unvaccinated in 2015-16 stratum. It is anticipated that a percentage of the randomized study subjects may not complete the study.

Participants will be randomized to receive IIV3, HD-IIV3, or aIIV3 at their baseline study visit to ensure an appropriate minimum number of subjects receive high dose and adjuvanted vaccines in 2016-17 for analyses. Participants will be grouped according to vaccination type (standard dose, high dose or adjuvanted vaccine) received during the 2016-17 season (**Table 1**). All subjects enrolled in Fall 2016 will have a baseline serum blood draw, a second serum blood draw 28 (±5) days after vaccination and 6 months after vaccination in each season (2016-17 and 2017-18). In Fall 2017, participants who received HD-IIV3 and aIIV3 in Fall 2016 will receive the same vaccine type. Participants who received IIV3 will be randomized to receive HD-IIV3, aIIV3, or RIV4 for the 2017-18 season.

An additional 30 adults who received IIV3 in the 2016-2017 season will be recruited in Fall 2017. Newly recruited participants will be randomized to receive HD-IIV3, aIIV3, or RIV4 for the 2017-18 season and will have serum blood draw before vaccination, 28 (±5) days after vaccination and 6 months after vaccination.

Table 1. 2016-17 Vaccine Randomization & Blood Draw Scheme with Proposed ID numbering

	Group	2016-17 Vaccine	2017-18 Vaccine	Total
65-74 years	A	2016-17 HD-IIV3	2017-18 HD-IIV3	60
	B	2016-17 aIIV3	2017-18 aIIV3	60
	C1	2016-17 IIV3	2017-18 high dose trivalent inactivated influenza vaccine	30
	C2	2016-17 IIV3	2017-18 aIIV3	30
	C3	2016-17 IIV3	2017-18 RIV4	30
		Total		210

All participants will be contacted weekly throughout the influenza season to monitor for influenza-like illness and, if exhibiting symptoms, they will be scheduled for nasal and throat swabs to be tested for influenza.

Study follow-up will span approximately two years from the first dose for participants enrolled in the first year. Study follow-up will span approximately 6 months from their enrollment date for study subjects recruited in Fall 2017.

3.2 Study Endpoints

3.2.1 Primary Endpoints

- Hemagglutinin inhibition antibody levels specific for vaccine strains and representative strains of circulating influenza viruses. Endpoints based on these data will include geometric meant titers (GMT), mean fold rise (MFR), seroresponse rate (SRR), and seroprotection rate (SPR).
    - Seroresponse rate defined as the proportion of subjects with paired samples with prevaccination HI titer <1:10 and postvaccination (day 28) HI titer ≥1:40 or at least a 4-fold increase in HI from prevaccination HI titer ≥1:10.
    - Seroprotection rate defined as the proportion of subjects with postvaccination HI titer ≥1:40.

3.2.2 Secondary Endpoints

- Virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers specific for vaccine strains and representative strains of circulating influenza viruses. Endpoints based on these data will include geometric meant titers (GMT) and mean fold rise (MFR).
- RT-PCR confirmed influenza

3.3 Randomization Procedure

We will use stratified block randomization to ensure more even distribution of subjects with prior receipt of 2015-16 influenza vaccine. Subjects will be stratified based on receipt of 2015-

16 influenza vaccine. Within each group (vaccinated or unvaccinated in 2015-16), sequential enrollment numbers will be randomly assigned to each vaccine group in random blocks ranging from four to eight. This will ensure proper distribution of subjects across vaccine group assignment.

In Fall 2017, only participants who received IIV in 2016-17 will be randomized. We will use stratified block randomization and subjects will be stratified based on participation in this study during the 2016-17 season (previously participated or new recruit).

This is an open-label study, therefore the vaccine type will be known to the participant. Trained research staff will prepare and administer the influenza vaccine. Randomization to vaccination type will occur at the participant's first study visit after eligibility is confirmed and informed consent obtained. Those enrolled in year 1 who receive IIV3 will be randomized a second time at their fourth visit.

3.4 Study Duration

The maximum duration of a subject's participation in the study is approximately two years.

4 VACCINES

All subjects will receive an FDA approved and recommended influenza vaccine in each season. Any individual with a contraindication to influenza vaccination will be unable to participate in the study. The four influenza vaccines that will be used in this study are 1) IIV3 (Fluvirin, Seqirus), 2) HD-IIV3 (Fluzone High-Dose, Sanofi Pasteur), 3) aIIV3 (Fluad, Seqirus) and 4) RIV4 (FluBlok, Protein Sciences). Vaccines will be stored and administered according to the manufacturer's package insert (Fluvirin: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM123694.pdf, Fluzone High-Dose: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM305079.pdf, Fluad: http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/UCM474387.pdf)FluBlok: https://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm524684.pdf

5 SELECTION OF STUDY SUBJECTS

5.1 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible to participate:

1) Age 65 through 74 years at the time of enrollment and are ambulatory and live in Marshfield, Wisconsin or surrounding communities.
2) Willing and able to give informed consent prior to study enrollment.
3) Able to comply with study requirements.

5.2 Exclusion Criteria

Subjects will be excluded if they fulfill any of the following criteria:

1) Prior receipt of 2016-17 (or 2017-18 for participants recruited in Fall 2017) influenza vaccine.
2) Current participation in another clinical trial.
3) Presence of a contraindication to influenza vaccines as described by the Advisory Committee on Immunization Practices.

6 STUDY PROCEDURES

6.1 Enrollment Period

Enrollment in this study will begin when influenza vaccine is available through the Marshfield Clinic, typically in August or September 2016, and will continue until the target sample size for each group is reached or all eligible adults have been contacted with the goal of completing all baseline study visits by the end of October 2016. The second enrollment period for the additional 30 subjects will follow the same recruitment procedures in Fall 2017.

6.2 Recruitment

A recruitment letter that includes information on the FDA approved influenza vaccines used in this study will be sent to a sample of individuals aged 65-74 years who receive outpatient care from Marshfield Clinic facilities. Additional mailings will be sent, as needed, to additional patients who receive outpatient care from Marshfield Clinic facilities in batches until the target sample size in each group is reached. Marshfield Clinic patients who do not receive a recruitment letter but would like to participate in the study may participate provided they meet study eligibility criteria.

The letter will be followed by telephone contact within 10 days to assess interest, screen for contraindications to any vaccine, answer questions, and schedule a pre-vaccination appointment for those who have no contraindications and give initial verbal consent. Study visits will be scheduled at convenient times for the enrollee and to allow for efficient processing of blood samples (see additional detail in laboratory methods).

6.3 Study Visit Procedures

Participants enrolled in the first season will have 6 scheduled study visits over an approximately 18 month period. At each visit, all participants will have a blood draw (20 mL [4 teaspoons]) for

evaluation of serologic response. Influenza vaccine will be administered at visit 1 and 4, prior to each influenza season. Participants will have a maximum of 6 blood draws during the study period.

Participants enrolled in Fall 2017 will have 3 scheduled study visits over an approximately 6 month period. At each visit, participants will have a blood draw (20 mL [4 teaspoons]) for evaluation of serologic response. Influenza vaccine will be administered at the enrollment visit (Study Visit 4).

6.3.1 Visit 1: Enrollment and Vaccination – Approximately 60 minutes

Research staff will meet with eligible individuals to review study requirements and obtain written consent. A brief questionnaire may be administered to obtain additional information regarding demographics and relevant exposure and medical history. We will also request permission from individuals to access their electronic medical record in order to obtain data related to medical history, medication use, and vaccination history. The remaining study visits will be scheduled.

6.3.1.1 Blood draw

All participants will have a baseline blood draw for measurement of antibody titers (20 mL [4 teaspoons]), hemagglutination inhibition [HI] and other assays (see below). Serum from the samples will be prepared, frozen, and tested per current protocol (see laboratory methods). Additional measures of humoral immune function may also be examined.

6.3.1.2 Influenza vaccine

Individuals will receive either 1) IIV3, 2) HD-IIV3, or 3) aIIV3 according to a pre-assigned randomization scheme. Trained research staff will administer all vaccines.

Individuals with fevers will be rescheduled regardless of the vaccine to be received. Vaccinations will be recorded in the electronic immunization registry, and vaccine information sheets will be provided.

6.3.2 Visit 2: Day 28 Post-Vaccination, Year 1 – Approximately 30 minutes

All participants will have a visit with blood draw at 28 (±5) days (20 mL [4 teaspoons]) for evaluation of serologic response. Research staff will also ask for the best time to call during the illness surveillance phase of the study.

6.3.3 Visit 3: 6-month Post-Vaccination, Year 1 – Approximately 30 minutes

All participants will be scheduled to collect a 6-month post-vaccination serum specimen at approximately 182 (±10 days) days following receipt of the 2016-17 influenza vaccine to evaluate serologic response. The blood draw will be 20 mL (4 teaspoons).

6.3.4 Visit 4: Year 2 Vaccination – Approximately 40 minutes/Enrollment and Vaccination of New Recruits – Approximately 60 minutes

6.3.4.1 Year 2 Vaccination

All participants who received the 2016-17 high dose trivalent inactivated influenza vaccine and 2016-17 aIIV3 at enrollment will receive the same vaccine type at this visit (for the 2017-18 season). Participants who received 2016-17 standard dose inactivated influenza vaccine at enrollment will be randomized to receive high dose trivalent inactivated influenza vaccine, aIIV3 or RIV4 at this visit (for the 2017-18 season). Prior to vaccination, all participants will have a blood draw to evaluate serologic response. The blood draw will be 20 mL (4 teaspoons).

Individuals with fevers will be rescheduled regardless of the vaccine to be received. Vaccinations will be recorded in the electronic immunization registry, and vaccine information sheets will be provided.

6.3.4.2 Enrollment and Vaccination of New Recruits

Research staff will meet with eligible individuals to review study requirements and obtain written consent. A brief questionnaire may be administered to obtain additional information regarding demographics and relevant exposure and medical history. We will also request permission from individuals to access their electronic medical record in order to obtain data related to medical history, medication use, and vaccination history. The remaining study visits will be scheduled.

All participants will have a baseline blood draw for measurement of antibody titers (20 mL [4 teaspoons]), hemagglutination inhibition [HI] and other assays (see below). Serum from the samples will be prepared, frozen, and tested per current protocol (see laboratory methods). Additional measures of humoral immune function may also be examined. Participants will receive either 1) HD-IIV3, 2) aIIV3, or 3)RIV4 according to a pre-assigned randomization scheme. Trained research staff will administer all vaccines.

Individuals with fevers will be rescheduled regardless of the vaccine to be received. Vaccinations will be recorded in the electronic immunization registry, and vaccine information sheets will be provided.

6.3.5 Visit 5: Year 2, 1-month Post-Vaccination – Approximately 30 minutes

All participants will have a visit with blood draw at 28 days (20 mL [4 teaspoons]) for evaluation of serologic response.

6.3.6 Visit 6: Year 2, 6-month Post-Vaccination – Approximately 30 minutes

All participants will be scheduled to collect a 6-month post-vaccination serum specimen in year 2 at approximately 182 (±10 days) days following receipt of the 2017-18 influenza vaccine to evaluate serologic response. The blood draw will be 20 mL (4 teaspoons).

6.3.7 Illness Surveillance

During both influenza seasons, enrolled subjects will be followed prospectively to monitor for influenza illness. Participants will receive a weekly call from an interviewer to ask approximately 5 screening questions regarding presence of influenza like illness or respiratory symptoms including cough and duration of illness. A call list will be generated each week. The timing of the influenza season (surveillance phase) will depend on observations of influenza activity in the community and state-wide. We anticipate the surveillance period to be approximately November 1 through April 1 of each season. A reminder letter regarding the start of this phase of the study will be sent to enrollees approximately two weeks prior to the start of telephone calls.

"New onset" will require a period free of symptoms, or with baseline symptoms, and at least 14 days from onset of previous illness to differentiate an episode from any prior illness. When a new acute respiratory illness is reported and confirmed, an appointment to obtain a combined nasal and oropharyngeal (throat) swab for influenza testing will be scheduled within 7 days of illness onset if a participant reports an acute respiratory illness. Subjects will be asked to come to the clinic to obtain the respiratory sample, but home visits by research staff may also be available for subjects who are unable to come to the clinic.

Subjects will continue to receive weekly telephone calls for ascertainment of respiratory symptoms. Subsequent weekly calls for persons with recently reported acute respiratory illness will document resolution, or return to baseline, of symptoms.

6.4 Data Collection

In addition to data collected through the enrollment survey, we will also electronically abstract data on vaccination and medical history from the immunization registry and electronic medical record.

6.4.1 Influenza Vaccination Status

The availability and types of influenza vaccines vary from season to season. Vaccination status and vaccine types received will be obtained for prior seasons for all participants.

We have previously validated the use of the local immunization registry at Marshfield Clinic, RECIN, to correctly categorize receipt of influenza vaccine.[18] Since RECIN exchanges records with the Wisconsin Immunization Registry, all vaccines administered by public health immunization providers will be identifiable. Immunization records have sufficient detail to

distinguish inactivated vaccine types, and to distinguish different manufacturers and lot numbers.

6.4.2 Medical Record Review

We may abstract medical records for participants. The goal of the abstraction will be to identify potential risk factors for no or low immune response to vaccination or vaccine failure. The elements that may be extracted electronically from medical records include: dates of medical visits for acute respiratory illness, tests and procedures performed, high risk conditions, influenza vaccination status in prior seasons, and patient demographics.

7 SPECIMEN COLLECTION AND HANDLING

All blood samples will be collected at the Marshfield Clinic Central Laboratory by trained staff. The blood samples will be collected using Vacutainer tubes, with uniformly colored tops according to type of processing (e.g., red tops for serum), and appropriately labeled with a uniform scheme (including study ID and collection time point). Up to 20 mL blood will be collected at each visit.

Nasal and throat swabs will be collected by research staff who have been trained on the proper technique for collection of nasal and throat swabs. Research staff must demonstrate use of proper techniques before they are allowed to collect samples by study participants. Sample quality (human RNase P) will be monitored by RT-PCR to ensure adequate sample collection procedures.

To support better understanding of the immune response following influenza vaccination, specimens will be banked for future studies that might involve respiratory illnesses.

7.1 Serum

For serum collection, whole blood drawn in a tube without anticoagulant will be left at room temperature for a minimum of 30 minutes and maximum of 2 hours. The samples will then be placed at 4° C. Samples will be processed within 24 hours of collection, and ideally the following morning for these afternoon/evening appointments. Tubes will be placed in a table-top centrifuge and serum will be clarified by centrifugation at 3000 rpm for 10 minutes at 4° C. Serum will be aliquotted into labeled, aliquot specific, cryovials with an adjustable pipettor. Aliquot size will be determined based on cross-site decisions. Serum will then be stored at -80°C in labeled fiberboard boxes and stored in the Marshfield Clinic Research Institute Integrated Research and Diagnostic Laboratory per current protocol until shipping. Once all participants have been enrolled and have completed all required blood draws, batched samples will be shared with collaborators at the CDC (or their designee) in order to conduct serologic assays.

7.2 Swabs

Combined nasal and oropharyngeal (throat) swabs for influenza testing will be collected by trained research coordinators. The nasal swab is quick and painless, and we have found that it is acceptable and well-tolerated. The nasal swab is collected by inserting a large Dacron or rayon tipped swab into one nostril to a depth of 1-2 cm. It is rotated gently against the septum for 3-5 seconds and then withdrawn. The throat swab is obtained by swabbing both tonsils and the posterior oropharynx. A sterile polyester-tipped applicator will be used for swab collection. The swab tips are immediately placed in M4-RT viral transport media and labeled. Specimens will be refrigerated or held on ice until they are transported to the laboratory on the same day.

8 LABORATORY METHODS

Blood samples from each study subject will be drawn for influenza serology testing at each of the six scheduled study visits. Hemagglutination inhibition (HI) antibody titers will be determined for all subjects at all time-points. Testing for virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers will depend on HI results. Serologic parameters will include geometric mean titers (GMT), mean fold rise (MFR), seroresponse rate (SRR), and seroprotection rate (SPR) by vaccine group. Additional measures of humoral immune function may also be examined.

A brief description of each serological assay is provided in the following sections.

8.1 Hemagglutination Inhibition Antibody Testing

Serum samples from all subject collected at each of the 6 study visits will be tested by hemagglutination inhibition (HI) assays to estimate antibody titers against the 2016-17 vaccine strains and representative strains of circulating influenza viruses that are antigenically or genetically different during 2016-17 season in year 1 (visits 1, 2, 3 and 4) and 2016-17 and 2017-18 vaccine strains and representative strains of circulating influenza viruses that are antigenically or genetically different during 2017-18 season in year 2 (visits 4, 5, and 6) using standard techniques.[19] Each serum sample is tested in duplicate and the final titer is the geometric mean titer (GMT) of the duplicate titers. HI testing will be conducted at CDC or by a CDC-designated laboratory.

8.2 Microneutralization Antibody Testing

Virus neutralization titers will be determined by a standard microneutralization (MN) assay, which is a highly sensitive and specific assay for detecting virus-specific neutralizing antibodies to influenza viruses.[19]Testing for MN titers will depend on the circulating virus for each season. Each serum sample will be tested in duplicate and the final titer is the geometric mean titer (GMT) of the duplicate titers. MN testing will be conducted at CDC.

8.3 Neuraminidase Antibody Testing

Testing to assess neuraminidase antibody mediated inhibition of influenza viruses with the neuraminidase inhibition assay (NAI) will depend on HI responses. Neuraminidase inhibition assays will be conducted at CDC using standard techniques.[19]

The NAI assay, also known as the enzyme-linked lectin assay, measures antibodies that inhibit the enzymatic activity of viral neuraminidase, utilizing a reassortant influenza virus with a mismatched hemagglutinin (H6 subtype), to avoid interference by HA-specific antibodies, and neuraminidase from vaccine reference viruses. The percent inhibition of neuraminidase enzymatic activity at each serum dilution is calculated by comparison with values from virus control wells (virus but no serum); end-point NAI titers are calculated as the reciprocal of the highest dilution with at least 50% inhibition.

8.4 Antibody Dependent Cellular Cytotoxicity (ADCC) Analysis

A modified influenza HA-specific antibody-dependent cell-mediated cytotoxicity natural killer (NK) cell activation assay will be quantified using a modified ADCC natural killer (NK) cell activation assay.[20] Testing for ADCC will depend on baseline responses as measured by HI. Serially diluted human serum samples are added to wells containing recombinant HA and mixed with a human NK cell line and mouse anti-human CD107a antibody to measure CD107a expression on NK cells stimulated by ADCC antibodies present in sera. The results are expressed as endpoint titers, for example, the highest serum dilution that achieved the 3% of the arbitrary threshold. Each serum sample is tested in duplicate and the final titer is the geometric mean titer (GMT) of the duplicate titers. ADCC testing will be conducted at CDC.

8.5 RT-PCR

Nasal and oropharyngeal (throat) swabs will be combined and tested using real-time reverse transcription polymerase chain reaction (RT-PCR) by staff from Marshfield Clinic Research Institute Integrated Research and Diagnostic Laboratory. CDC primers, probes, and procedures will be used for this assay. This PCR assay was developed and evaluated by CDC. Standard operating procedures for RT-PCR have been developed and will be followed. Total nucleic extractions will be performed using the Roche MagNA Pure Total Nucleic Acid Kit (Roche Diagnostics, Indianapolis, IN) on 200 µl of clinical samples. This is a high throughput, automated magnetic bead technology. Real-time RT-PCR will be performed on nucleic acid extracts using the LightCycler® Real-Time PCR System (Roche Diagnostics, Basel, Switzerland). The RT-PCR assay is a TaqMan® based real-time detection of the matrix protein (M1) of influenza A and the non-structural protein 1 (NS1) of influenza B. Both have been shown to be highly conserved, representing effective targets for detection. For each sample, the initial RT-PCR amplifies nucleic acid for type A and type B. The human RNase P gene primer and probe set serves as the internal positive control for human RNA. Samples positive for influenza A will be retested for subtype identification. These include primers for H1N1pdm09, and H3. Samples positive for influenza B will be further tested for B lineage.

Standard laboratory safeguards have been incorporated into the protocol to decrease the risk of carryover amplicon contamination. These include the use of uracil glycosylase, three separate unidirectional work flow areas, and laboratory surface decontamination products to eliminate any residual DNA/RNA.

9 PARTICIPANT COMPENSATION

Participants will be compensated for the time and effort required to participate in this study. Each participant will receive a $50 gift certificate for each blood sample provided (up to $300 total), and a $20 gift certificate for each combined nasal and oropharyngeal swab sample provided.

10 SHARING OF LABORATORY SPECIMENS AND DATA

Marshfield Clinic Research Institute will share participant samples (serum and swabs) with CDC, or a laboratory designated by CDC. Record review and vaccination history data will also be shared with CDC, with appropriate privacy measures in place.

11 DATA MANAGEMENT

11.1 Recording and Collection of Data

Microsoft Access will be used for enrollment and data management. Research coordinators will carry tablet computers to access the Access database on the secure wireless network. Staff will use Access to assess eligibility, schedule and enroll patients, and track study visits. Microsoft Access has robust procedures to ensure accurate and complete data collection. Access will require documentation of informed consent, responses to all inclusion and exclusion criteria, specimen collection dates, and vaccination information (date, type, manufacturer, and lot number). Radio buttons, pick lists and pre-programmed data checks will be used to ensure valid data entry. Access records will be exported to SAS files for final analyses. RECIN immunization records will be merged with Access enrollment records to define exposure (vaccination) status.

Reports will be generated on a regular basis to track recruitment, enrollment, and study visit completion and shared with collaborators during bi-weekly conference calls.

11.2 Data Quality Assurance

Study data will be entered by trained study staff. The study programmer will monitor and review the study databases regularly. The study programmer will run a series of quality checks and validation to look for missing, incomplete, or inconsistent data. Any identified data issues will be reconciled with study staff prior to finalization of the dataset and analysis.

12 STATISTICAL CONSIDERATIONS

12.1 General

Demographic and baseline characteristics will be summarized by vaccine group for all subjections. Continuous variables will be presented by summary statistics (e.g., mean, and standard deviation (SD)) for non-immunogenicity endpoints, and geometric means and their 95% confidence interval for the immunogenicity endpoints. Categorical variables will be presented by frequency distributions; i.e., frequency counts and percentages for the non-immunogenicity endpoints, and the percentages and their 95% confidence intervals for the immunogenicity endpoints.

12.2 Analyses Addressing Objectives

12.2.1 Analyses of Primary Objectives

After log transformation of the antibody titers, geometric mean antibody titers (GMT) pre- and post- vaccination will be compared within subjects by vaccine type. For the primary aim, adults receiving high dose trivalent inactivated influenza vaccine will be compared with those receiving aIIV3.

The mean and standard deviation of log titers will be calculated. Linear mixed-effects models will be used to adjust for variables such as age, given the potential for small differences in age and the known difference in GMT by age, baseline antibody titer and prior season vaccination status. The mean fold rise (MFR), defined as the mean of the ratio of post vaccination GMT and pre-vaccination GMT for each subject, between the groups will be computed from mixed-effects model along with its associated 95% confidence interval (CI). For the declaration of non-inferiority of GMT between groups, the lower bound of the adjusted 95% CIs for the GMT ratios will be determined for each strain.

12.2.2 Analyses of Secondary Objectives

Analyses of virus neutralizing antibody titers, neuraminidase antibody mediated inhibition titers, and anti-human CD107a antibody titers will be similar to HI data described above. Secondary analyses will also include comparisons of GMT for HI and neuraminidase inhibition assays, seroconversion rates, and ADCC measures by prior season vaccination and vaccine type in the current season.

If vaccine failures (RT-PCR confirmed influenza infection) occur, we will assess factors associated with failure using logistic regression, including vaccine type.

12.3 Power Considerations

Study sample size was based on available resources. For the analysis on HI titers, a sample size of 60 per group will provide over 90% power using a one-sided, two-sample t-test to conclude that one vaccine group is superior as defined by rejecting a null hypothesis of equality if the GMT values differ by approximately 20%.

13 HUMAN SUBJECTS

13.1 Informed Consent

Participants in this study will provide written consent and/or assent for specimen collection, influenza vaccination, and medical record review. Further details regarding data privacy and protection of human subjects are provided in the IRB application document.

14 REFERENCES

1. Assaad FA, Reid D. Some factors influencing mortality from influenza. Bull World Health Organ 1971;45:113-7.
2. Lui KJ, Kendal AP. Impact of influenza epidemics on mortality in the United States from October 1972 to May 1985. Am J Public Health 1987;77:712-6.
3. Barker WH, Mullooly JP. Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths. JAMA : the journal of the American Medical Association 1980;244:2547-9.
4. Barker WH, Mullooly JP. A study of excess mortality during influenza epidemics in the United States, 1968-1976. Am J Epidemiol 1982;115:479-80.
5. Sprenger MJ, Van Naelten MA, Mulder PG, Masurel N. Influenza mortality and excess deaths in the elderly, 1967-82. Epidemiol Infect 1989;103:633-41.
6. Eickhoff TC, Sherman IL, Serfling RE. Observations on excess mortality associated with epidemic influenza. JAMA 1961;176:776-82.
7. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289:179-86.
8. Orsi A, Ansaldi F, de Florentiis D, et al. Cross-protection against drifted influenza viruses: options offered by adjuvanted and intradermal vaccines. Human vaccines & immunotherapeutics 2013;9:582-90.
9. Aspinall R, Del Giudice G, Effros RB, Grubeck-Loebenstein B, Sambhara S. Challenges for vaccination in the elderly. Immun Ageing 2007;11:9.
10. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond) 1972;70:767-77.
11. Govaert TM, Sprenger MJ, Dinant GJ, Aretz K, Masurel N, Knottnerus JA. Immune response to influenza vaccination of elderly people. A randomized double-blind placebo-controlled trial. Vaccine 1994;12:1185-9.
12. Nabeshima S, Kashiwagi K, Murata M, Kanamoto Y, Furusyo N, Hayashi J. Antibody response to influenza vaccine in adults vaccinated with identical vaccine strains in consecutive years. Journal of medical virology 2007;79:320-5.
13. Sasaki S, He XS, Holmes TH, et al. Influence of prior influenza vaccination on antibody and B-cell responses. PloS one 2008;3:e2975.
14. Ng S, Ip DK, Fang VJ, et al. The effect of age and recent influenza vaccination history on the immunogenicity and efficacy of 2009-10 seasonal trivalent inactivated influenza vaccination in children. PloS one 2013;8:e59077.
15. Keitel WA, Cate TR, Couch RB. Efficacy of sequential annual vaccination with inactivated influenza virus vaccine. American journal of epidemiology 1988;127:353-64.
16. Keitel WA, Cate TR, Couch RB, Huggins LL, Hess KR. Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five year period. Vaccine 1997;15:1114-22.

17. DiazGranados CA, Dunning AJ, Robertson CA, Talbot HK, Landolfi V, Greenberg DP. Effect of Previous-Year Vaccination on the Efficacy, Immunogenicity, and Safety of High-Dose Inactivated Influenza Vaccine in Older Adults. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2016;62:1092-9.
18. Irving SA, Donahue JG, Shay DK, Ellis-Coyle TL, Belongia EA. Evaluation of self-reported and registry-based influenza vaccination status in a Wisconsin cohort. Vaccine 2009;27:6546-9.
19. Manual for the laboratory diagnosis and virological surveilance of influenza. WHO, 2011. at http://apps.who.int/iris/bitstream/10665/44518/1/9789241548090_eng.pdf.)
20. Zhong W, Liu F, Wilson JR, et al. Antibody-Dependent Cell-Mediated Cytotoxicity to Hemagglutinin of Influenza A Viruses After Influenza Vaccination in Humans. Open forum infectious diseases 2016;3.