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Impact of Repeated Prior Influenza Vaccination on Serum Hemagglutinin Inhibition Antibody Response to B/Victoria/Brisbane/60/2008-like virus in 2010-11 Inactivated Influenza Vaccine and Prior Seasonal Victoria and Yamagata Influenza B Lineage Components among Healthcare Personnel

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Epidemiology

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Emory University

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Abstract

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Methods: Hemagglutination inhibition antibody (HI) assays were collected preseason and ~30 days post-vaccination from a prospective healthcare personnel (HCP) cohort. Eligible participants had confirmed medical and vaccination records for four years and received the 2010-11 trivalent inactivated influenza vaccine (IIV3) containing B/Brisbane/60/2008-like virus (B/Victoria). Preseason and post-vaccination geometric mean titers (GMTs), geometric mean ratio (GMR), and fold-change were assessed for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria) response adjusted for age, sex, race, education, household size and hospital care responsibilities.

Results: All three viruses had post-vaccination fold change results inversely associated with the number of prior vaccinations and only B/Florida experienced a significant, direct, association for preseason GMT. B/Brisbane and B/Malaysia experienced a significant increase in adjusted GMR among those with no prior vaccinations (GMR = 1.61 and 1.42, respectively) compared to having received at least one prior vaccination. B/Florida exhibited a similar increase in adjusted GMR for no prior vaccinations (GMR = 1.18) and was significantly higher than HCP that received 2-4 prior vaccinations.

Conclusions: Our findings suggest that a single prior IIV3 vaccination reduces immune response and cross-reactivity of influenza B virus with non-significant differences between one and four prior vaccination responses. Ultimately, more research into specific B vaccine component response and cross-reactivity to other B viruses may be needed to optimize immunogenicity and vaccine effectiveness among HCP and other repeated vaccinees.

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Chapter I: Background and Literature Review

Introduction

Annual, seasonal cases of influenza are due to the co-circulation of type A and B influenza viruses. In contrast, influenza C viruses tend to remain endemic as sporadic sources of relatively mild disease. Influenza A and B viruses are known to be a common source of human respiratory infections, illness, and death. Type A, with circulating subtypes H1N1 and H3N2, are of particular concern due to their pandemic potential and capability of producing serious illness [1]. Influenza B, although not associated with pandemics, has been known to cause epidemics and presents a significant burden of influenza on society and healthcare systems. In the mid-1970s, influenza B began to diverge into two antigenically distinct genera, B/Victoria and B/Yamagata [2]. Since this divergence, the two lineages have exchanged dominance of seasons as well as geographical location. The frequent fluctuation of transmission between these two viruses require recurrent updates to immunization recommendations. The largely distributed trivalent vaccine includes only one of the lineages of influenza B virus and thus requires sufficient estimation as to which lineage will be most prevalent in a given season.

Burden of Disease

In the northern and southern hemispheres, influenza takes on a season that spans the colder months of the calendar year. This generally equates to September through February of the subsequent year for the northern hemisphere and February through September for the southern hemisphere [3, 4]. There is a less defined season for countries in the equatorial region which

have low but maintained influenza transmission throughout much of the year Influenza B maintains a significant portion of the overall influenza burden despite being less virulent than type A. In the 2015-2016 season in the United States, the total influenza positive tests reported to the Centers for Disease Control and prevention (CDC) by public health laboratories had 13.42% B/Yamagata, 6.20% B/Victoria, and 10.09% uncharacterized B lineage. Cumulatively, influenza B represented nearly 30% of all positive reported influenza tests for the season. Influenza B accounted for approximately 25% of influenza-associated hospitalizations and 31% of influenza-associated pediatric fatalities [5].

The cumulative burden for influenza in the United States alone is significant. The approximate five season range, spanning influenza seasons of 2010-2011 to 2014-2015, there is an estimated 9.2-35.6 million influenza illnesses that correspond to 4.3-16.7 million medical visits. 140,000-710,000 per 100,000 population are hospitalized for influenza and 4,000-20,000 excess deaths are attributable to pneumonia and influenza [6]. In pediatric cases of influenza specifically, inpatient medical costs are approximately 3,990 USD per patient and the average cost for each emergency department visit is approximately 730 USD [7]. The extensive burden of influenza in the population, coupled with the cost-to-treat each patient, represents a substantial strain on the healthcare system.

While these clinical estimates provide a degree of context for the burden of influenza, there are still gaps in knowledge. Influenza is a challenging illness to diagnose due to its similarity in clinical symptoms as other respiratory diseases. In many instances, influenza is combined into other frequent respiratory illnesses like bronchitis, respiratory syncytial virus (RSV), or adenovirus as influenza-like-illness (ILI). Without proper diagnostic testing of the viral pathogen, a case of ILI will go unregistered as laboratory-confirmed influenza. The

laboratory testing itself poses issues in validity due to the spectrum of instrumentation. Table **1** displays a variety of common laboratory testing methods and their quality in terms of sensitivity and specificity.

Table 1. Influenza diagnostic testing methods [8].

Diagnostic Test	Time to Results	Sensitivity	Specificity
Polymerase Chain Reaction (PCR)	2 hours	High	High
Immunofluorescence	2-4 hours	Moderately high	High
Rapid Diagnostic Tests	< 30 minutes	Low-moderate	High
Viral Culture	>2 days	Moderate-high	Highest

Since the 2009 A(H1N1)pdm09 pandemic, PCR has emerged as the gold standard of influenza diagnostics. Although it is still expanding in use, other diagnostic tests are still frequently used. Rapid diagnostic tests have been of particular importance due to their fast rate of results and inexpensive, portable design. However, these tests substantially underperform other established diagnostic tests which may lead to poor incidence reporting. The use of unreliable influenza confirmation tests may lead to under reporting of influenza illness during medical visits. Having a limited understanding of the true burden of disease poses complications to interventions and assessment of risks in populations.

Epidemiology

Influenza illness may occur in every portion of the general population, but it does disproportionately impact some groups more than others. Children and older adults are of particular concern when it comes to disease severity and transmission. Table **2** outlines estimated influenza-associated hospitalization rates by age groups using a negative binomial regression model in the United States. These values are mean estimates and confidence intervals (CI) spanning from the 1993-1994 season to 2007-2008 season [9].

Table 2. Mean estimates of influenza-associated hospitalization rates (per 100,000) in the United States from 1993-2008.

Age Group	**Mean Rate per 100,000 (95% CI)**
< 1 year	151.0 (105.3-659.6)
1-4 years	38.8 (24.1-213.2)
5-49 years	16.8 (9.8-58.4)
50-64 years	65.6 (35.0-270)
≥65 Years	309.1 (186-1103.7)
All ages	63.5 (37.5-236.6)

Rates of influenza-associated hospitalizations are substantially greater for those aged less than one year and those sixty-five years and older. Older adults, ages sixty-five years and older,

are of particular concern for influenza mortality. From 2010-2013, 71-85% of all influenza deaths in the United States occurred among older adults ages sixty-five years and older [10]. Age is thus an inherent risk factor in severe influenza illness, with interventions aimed at these population age groups.

Although young to middle aged adults are at lower risk for severe influenza, pregnant women are among those at high risk of adverse outcomes. Influenza illness during pregnancy has been linked to more severe symptoms, potential pregnancy complications, and neonate transmission [11, 12, 13].

Chronic conditions such as chronic lung disease, asthma, diabetes, or cardiovascular disease pose an increased risk for severe influenza illness [14, 15]. Immunosuppression due to illnesses such as HIV or cancer also subject the individual to greater complications due to influenza [16].

Immunization Practice

One of the most efficient and effective means of protecting the general population and at-risk individuals is through routine seasonal influenza vaccination. Seasonal influenza immunizations undergo an extensive process to prepare for the following year's formulation. Data are collected from the Global Influenza Surveillance and Response System (GISRS), a multi-platform international surveillance network that includes National Influenza Centers (NICs), World Health Organization Collaborating Centers (WHO CCs), and other reference laboratories. GISRS collects all submitted viral data, as well as epidemiologic and clinical findings, to provide a context for influenza type and subtype circulation. The types and subtypes are antigenically characterized by WHO CCs to evaluate the immune response from the surface

proteins of influenza viruses. In this manner, the more specific form of influenza virus may be ascertained. GISRS laboratories also performed serology studies to assess how well antibodies from vaccinated persons react to the circulating viruses. This information contributes to the assessment of vaccine effectiveness by the Global Influenza Vaccine Effectiveness (GIVE) Collaboration. Viruses are also genetically characterized to determine how certain genetic changes may impact the protection that is delivered by the vaccine type. The final stage in influenza circulation assessment is testing for antiviral resistance. Antiviral therapy is a popular method to reducing the severity and duration of influenza illness but viruses have the potential to develop resistance to current antiviral formulations. The Advisory Committee on Immunization Practices (ACIP) uses all of the collected information to recommend what virus types, subtypes, and formulations should be included in the following season's vaccine. This process is repeated twice a year, once for the northern hemisphere and once for the southern hemisphere vaccines due to their potential to need different formulas for protection [17].

Vaccines are frequently egg-inoculated due to the ease in which influenza viruses grow in egg-based conditions. Trivalent vaccines are the most distributed and include an A(H1N1) component, A(H3N2) component, and one B component, either B/Victoria or B/Yamagata depending on the annual ACIP recommendation. Quadrivalent vaccines are available to provide both forms of influenza B protection.

In the 2014-2015 United States influenza season, 59.3% of children ages six months to seventeen years and 43.6% of adults greater or equal to eighteen years received a seasonal influenza vaccine [18]. The first recommended influenza vaccine age, six months, to twenty-three months reached coverage of 74.6% with waning coverage in later years to the age of seventeen. For adults, greater than or equal to sixty-five years of age, immunization coverage

was 66.7%. The coverage of influenza vaccination increased from preceding years and has continued a trend of increasing spread. Although rates of vaccination are increasing by year, they still fall short of the Healthy People 2020 goal of 70% vaccination for the whole population [19]. Widespread vaccination is shown to minimize influenza transmission and severe illness particularly in high-risk groups.

Despite the growing coverage of influenza immunizations in the United States, vaccine effectiveness against influenza illness remains a challenge. Given the reformulation of vaccines each season, the opportunity to mismatch vaccine components to circulating viral agents renews each year. The 2010-2011 season experienced 40% vaccine effectiveness against influenza A and B, as well as B alone. The subsequent 2011-2012 season showed increased vaccine effectiveness of 50% for influenza A and B and 47% for B alone [20].

In the same study, from 2010-2013, influenza vaccines were estimated to be 47% (95% CI: 42-52%) effective against influenza illness at clinics throughout the United States. Influenza B, specifically, was determined to be 50% (95% CI: 43-57%) effective in prevention of B virus. Younger populations had more protection from vaccination with effectiveness at 51% (95% CI: 35-64%) and 52% (95% CI: 40-62%) for six month olds to five year olds and six year olds to seventeen year olds, respectively. A high-risk population group, those over fifty years old, experienced 37% (95% CI: -10-65%) effectiveness against influenza B virus. Given the vaccination coverage of 42-43% in the United States over these study years, the gap in prevention is apparent [21]. With roughly half of the population receiving influenza immunizations and just less than half of immunized persons having boosted immunity, a substantial susceptible population remains at risk for illness.

Chapter II: Manuscript

Title, Authors, Abstract

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Introduction: Seasonal influenza B is an important contributor to the overall burden of influenza during each season. There is recent evidence of lower vaccine effectiveness (VE) against influenza A for those vaccinated in prior seasons. However, this relationship has not yet been investigated for influenza B.

Methods: Hemagglutination inhibition antibody (HI) assays were collected preseason and ~30 days post-vaccination from a prospective healthcare personnel (HCP) cohort. Eligible participants had confirmed medical and vaccination records for four years and received the 2010-11 trivalent inactivated influenza vaccine (IIV3) containing B/Brisbane/60/2008-like virus (B/Victoria). Preseason and post-vaccination geometric mean titers (GMTs), geometric mean ratio (GMR), and fold-change were assessed for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria) response adjusted for age, sex, race, education, household size and hospital care responsibilities.

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Conclusions: Our findings suggest that a single prior IIV3 vaccination reduces immune response and cross-reactivity of influenza B virus with non-significant differences between one and four prior vaccination responses. Ultimately, more research into specific B vaccine component response and cross-reactivity to other B viruses may be needed to optimize immunogenicity and vaccine effectiveness among HCP and other repeated vaccinees.

Introduction

Vaccination against seasonal influenza in the United States has demonstrated to be an effective and cost-efficient method in reducing transmission and illness [22]. With increasing vaccination coverage and repeated use between seasons, the influence on prior vaccination and immunogenicity has increasing importance [23, 24, 25]. Several recent studies have observed that vaccine effectiveness (VE) against illness is modified by prior vaccination history [26, 27, 28, 29,30].

The prospective cohort study of healthcare personnel (HCP) in 2010-11 has previously been used to report lower HI response to the A(H1N1)pdm09 vaccine component for those who received the monovalent, inactivated A(H1N1)pdm09 vaccine during the previous year. Repeated Trivalent Inactivated Influenza Vaccine (IIV3) have also been associated with HI response for A(H3N2). Previous studies have largely been focused on type A influenza circulation instead of type B strains.

In this study, we will capture the impact of repeated IIV3 vaccinations, for up to four seasons, with HI response to B/Brisbane/60/2008-like virus (B/Victoria) and B/Florida/4/2006-like virus (B/Yamagata). We aim to determine whether a dose-response relationship between the number of prior vaccinations and serologic immunogenicity for working age adults.

Methods

Enrollment and Design

Serum samples collected for this study were from enrolled HCPs ages 18-65 years providing direct patient care at Scott and White Healthcare (SWH) in Temple, TX. Details on cohort

recruitment have been outlined previously [31]. The cohort enrolled approximately 40% of the HCP population at SWH with males and physicians refusing enrollment more frequently than female and non-physician healthcare professionals.

This study reduced the total sample to include only those with confirmed medical and vaccination records for four years beginning in July, 2006. A four-year time period before the 2010-11 season was determined due to record extraction limitations by the Institutional Review Boards (IRB), increased employment stability and improved thoroughness of employee records starting in 2006. Only those with confirmed seasonal IIV3 vaccination, not live-attenuated influenza vaccination (LAIV), for the 2010-11 season were included due to differences in immune response between the two vaccine types [32].

Preseason serum samples were collected in September and October (time 1). Post-vaccination serum samples were required to be collected between 14 and 60 days since IIV3 administration. Enrollees that developed acute respiratory illness were tested for influenza infection, and if confirmed positive by real time reverse transcriptase polymerase chain reaction (RT-PCR), were excluded from analysis.

Prior to study enrollment, the cohort protocol was submitted and approved by IRB to comply with 21 CFR 56. Analysis for this study was performed using de-identified to comply with exemption status for Emory IRB.

Composition of Vaccines

The 2010-11 IIV3 administered to HCP contained A/California/7/2009 pdm(H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 like virus strains. GlaxoSmithKline

manufactured two lots of IIV3 used in approximately 90% of those vaccinated at SWH. Novartis International and Sanofi Pasteur comprise the remaining 10% of confirmed vaccine manufacturers.

Supplement Table A provides IIV3 composition information for the previous four influenza seasons. Four IIV3 vaccines between the 2006-07 and 2010-11 season contained B/Victoria lineages with the 2010-11 and 2009-10 IIV3 contained B/Brisbane/60/2008-like virus and 2007-08 and 2006-07 IIV3 containing B/Malaysia/2506/2004-like virus. 2008-09 IIV3 contained B/Florida/4/2006-like virus (B/Yamagata).

Hemagglutination Inhibition Antibody Assay

Battelle Laboratory (Aberdeen, MD) performed HI tests in duplicate for B/Brisbane/60/2008 virus using methods [33] described previously [26]. A standard turkey erythrocyte was prepared with samples treated with receptor-destroying enzymes to remove nonspecific inhibitors. Nonspecific agglutinins were removed by serum absorption with packed turkey red blood cells. Serum was diluted 2-fold starting from 1:10 with the HI titer being the reciprocal of the serum dilution in the last well with complete HI. The geometric mean titer (GMT) from duplicate results was reported and an HI result less than ten considered to be five for GMT calculation.

Statistical Analyses

HI titer data was transformed to log base-2 to adjust for a highly left skewed distribution and then back-transformed to the original scale for interpretation [26, 34]. Repeated measures linear mixed models were fitted to estimate GMTs and geometric mean ratios (GMRs) due to pre- and

post-vaccine samples correlated within subjects. Compound symmetric covariance error structures were assumed for repeated measures within individuals.

GMTs were calculated by back-transforming the least squares mean estimates of log base-2 titer data. GMRs were calculated by back-transforming the difference in least squares mean estimates between post- and pre-vaccination logged base titers. GMRs are interpreted as simply the geometric mean ratio between post- and pre-vaccination titers. Multivariate models were adjusted *a priori* for age, sex and race [31]. A quadratic term for age was included to adjust for a nonlinear association. Education, household size and working in a hospital setting were used as additional covariates due to associations in the number of prior vaccinations or immune response based on previous research on this cohort [35]. A combined all-ages model adjusting for age and age squared was used unless effect modification by age was demonstrated. Associations between social variables and preseason GMT and post-vaccination fold change were assessed for possible inclusion in models. Detail on variable correlations with the number of prior vaccinations and immune response are provided in tables **1** and **2**.

An interaction term for time of sera draw between pre- and post-vaccination by the number of prior IIV3 vaccinations was estimated to assess the relationship between serologic immune response and the number of prior vaccinations. After adjustment for main effects and covariates, a statistically significant ($p < 0.05$) interaction term implied that immune response from vaccine was significantly modified by previous IIV3 exposure.

Vaccine exposure groups with non-overlapping 95% confidence intervals (CI) for GMTs and GMRs were considered statistically different. Partial eta squared ($\eta 2$) is reported for the mixed effect models to indicate variance in the outcome explained by the number of prior vaccinations

after excluding variance explained by covariates. Analyses were conducted using IBM SPSS Statistics 24 (Armonk, NY).

Results

Participant Characteristics

Of the initial 1,063 HCP in the cohort from Scott and White Hospital, a subset of 676 vaccinated HCP with preseason and post-vaccination sera in the 2010-11 influenza season were included for the study. Of these 676 HCP, 418 (62%) had confirmed medical records since 2006. HCP that received LAIV vaccination in any year since 2006, including the 2010-11 season, were excluded from the final sample (n=120). Three HCP were excluded for providing post-vaccination sera outside of the 14 to 63 day drawing window set forth in the study protocol. The final sample population of 295 HCP were used for analyses. A majority of the study participants were female, white, and non-Hispanic (Table **1**).

In the previous 4 influenza seasons, the mean number of prior vaccinations among HCP was 3. The number of prior vaccinations was directly associated with female HCP and increased with age. The number of prior vaccinations was lower among physicians, HCP working in a hospital, and inversely associated with the number of persons living in the participant's household.

Few factors were associated with preseason antibodies (Table **2**). Age was inversely associated with preseason GMT for B/Brisbane ($r = -.12$, $p < .05$) and B/Malaysia ($r = -.15$, $p < .05$). Although no significant associations between body mass index (BMI) and preseason GMT were observed, there were direct associations with BMI and post-vaccination fold change for B/Brisbane ($r = .16$, $p < .01$), B/Florida ($r = .15$, $p < .05$), and B/Malaysia ($r = .14$, $p < .05$).

Exploratory analyses for variables outside of *a priori* criteria were assessed for possible inclusion in the model. No other variables were discovered to have significant associations with preseason GMT and post-vaccination fold change.

Prior Vaccinations and B/Brisbane Response to 2010-11 IIV3 B/Brisbane Component

The number of prior vaccinations was associated with response to the 2010-11 IIV3 B/Brisbane component, as indicated by a significant correlation between prior vaccinations and fold-change in antibody titers between pre- and post-vaccination ($r = -.23$, $p < .001$) and a significant interaction effect between number of vaccinations and change in titers post-vaccination (partial $\eta 2 = .28$). This effect is driven by significantly higher adjusted GMR among HCP with no prior vaccinations (GMR = 1.61) compared to HCP with 1 or more prior vaccinations (GMR range = 1.10 – 1.16) (Table **4**). GMR for 1 through 4 prior vaccinations all have overlapping 95% confidence intervals suggesting similarities between these groups.

Although number of prior vaccinations was not significantly correlated with preseason GMT for B/Brisbane/60/2008 (Table **3**), HCP with no prior vaccinations had significantly lower pre-season GMT than those with 1 or more vaccinations (Table **4**). This small group of HCP started with a low GMT of 8.4 but increased to a GMT of 13.5 post-vaccination, which was significantly higher than the GMT results of those with 2-4 prior vaccinations (Figure **1**).

B/Brisbane preseason GMT and post-vaccination fold change was expected to be associated with the 2009-10 IIV3 due to B/Brisbane being that year's vaccine component (Appendix Table **A**). Preseason GMT was directly associated with the 2009-10 IIV3 ($r = .14$, $p < .05$) and inversely associated with post-vaccination fold change ($r = -.24$, $p < .001$). Although preseason IIV3 was

not associated with other specific IIV3, there were inverse associations with post-vaccination fold change and each year's vaccine (Table **3**).

Prior Vaccinations and B/Florida Cross-Reactivity to 2010-11 IIV3 B/Brisbane Component

The number of prior vaccinations was associated with cross-reactivity to the 2010-11 IIV3 B/Brisbane component by post-vaccination fold change ($r = -.13$, $p < 0.05$) and a significant interaction effect between number of vaccinations and change in titers post-vaccination (partial $\eta 2 = .22$). The effect appears to be driven by a higher adjusted GMR among HCP with no prior vaccinations (GMR = 1.18) compared to HCP with 1 or more prior vaccinations (GMR range = 1.04 – 1.09) (Table **4**). The GMR for those with no prior vaccinations was only significantly higher than HCP that received 2-4 prior vaccinations. HCP with 1-4 prior vaccinations all had GMR results indicating statistical similarities.

The number of prior vaccinations was significantly correlated with preseason GMT for B/Florida/4/2006 ($r = .15$, $p < .05$) (Table **3**). HCP with 0 prior vaccinations had a lower, but insignificant, preseason GMT of 9.9 compared to HCP with 1 or more prior vaccinations that ranged from 11.2 for HCP with 4 prior vaccinations to 10.4 for HCP with 1 prior vaccination (Table **4**). Post-vaccination GMT were statistically similar for all number of prior vaccination groups (Figure **2**).

We expected that the 2008-9 IIV3, containing B/Florida/4/2006, would have a significant association with B/Florida cross-reactivity. However, we did not see significant preseason GMT or post-vaccination fold change with this vaccine year (Table **3**). We observed significant direct associations for preseason GMT with the 2006-07 IIV3 ($r = .15$, $p < .01$) and 2007-08 IIV3 (r =

.11, $p < .05$). The only specific IIV3 associated with post-vaccination fold change was the 2009-10 IIV3 ($r = .13$, $p < .05$).

Prior Vaccinations and B/Malaysia Cross-Reactivity to 2010-11 IIV3 B/Brisbane Component

The number of prior vaccinations was associated with cross-reactivity to the 2010-11 IIV3 B/Brisbane component demonstrated by a significant inverse association with the number of prior vaccinations and post-vaccination fold change ($r = -.24$, $p < .001$) as well as a significant interaction effect between the number of prior vaccinations and change in post-vaccination titers (partial $\eta 2 = .21$). Adjusted GMR among HCP with 0 prior vaccinations (GMR = 1.42) was significantly higher than HCP with 1-4 prior vaccinations (GMR range = 1.09 – 1.14). GMR for those with 1-4 prior vaccinations were all statistically similar.

The number of prior vaccinations was not significantly correlated with preseason GMT for B/Malaysia/2506/2004 (Table **3**) but HCP with no prior vaccinations had significantly lower preseason GMT than those with 1 or 3-4 prior vaccinations (Table 4). HCP with 0 prior vaccinations increased to the highest, but not significantly higher, post-vaccination GMT of 12.5 compared to 11.2 for those with 4 prior vaccinations to 12.2 for those with 1 prior vaccination (Figure **3**).

B/Malaysia was the influenza B component in the 2006-07 and 2007-08 IIV3 (Appendix Table **A**) and were expected to have significant associations with preseason GMT and post-vaccination fold change. Only the 2009-10 IIV3 had a significant association with preseason GMT for B/Malaysia ($r = .13$, $p < .05$). Post-vaccination fold change was also inversely associated for each specific IIV3 (Table **3**).

Discussion

Serum HI titers for B/Brisbane and B/Malaysia following 2010-11 IIV3 vaccination varied for having 0 prior IIV3 vaccinations versus 1 or more IIV3 vaccinations, but minimal difference was observed for B/Florida. The magnitude of vaccine response, as the GMR, was statistically similar across groups of 1, 2, 3, or 4 prior vaccinations and significantly increased for those with 0 prior vaccinations (Figure **4**). Overall, GMT estimates for pre- and post-vaccination are minimal with no estimates of more than 15.

Our findings of 1+ prior vaccinations reduce GMR response compared to 0 prior vaccinations is not entirely consistent with previous results from McLean et al. This study suggests that vaccination has the highest effectiveness for those who are unvaccinated in the 5 prior seasons (VE = 75%, 95% CI = 50-87%) compare to those who were frequently vaccinated in the previous seasons (VE = 48%, 95% CI = 29-62%) [36]. This study determined a step-wise trend in vaccine effectiveness and number of prior vaccinations, but our study suggests statistical similarity for those with 1-4 prior vaccinations and a significant difference for those with no prior vaccinations. Thompson et al. similarly assessed A(H3N2) immune response from the number of prior vaccinations [35]. A(H3N2) followed an inverse exposure-response association between repeated vaccination and serologic response, while our influenza B results do not display this step-wise, inverse association.

Serologic findings do not necessarily correspond with clinical protection. While high HI titers do demonstrate an immune response due to vaccination, they do not imply clinical protection from illness. Conversely, low HI titers do not imply clinical risk to illness [37]. Nonetheless, individuals with previous exposure to influenza through vaccination experienced elevated HI titers at baseline compared to individuals without prior vaccinations in our study. Despite a

consistent trend of increased HI titers post-vaccination compared to pre-vaccination, the resulting titers remain low overall and do not imply a degree of seroconversion for protection against influenza illness.

Our findings are consistent with recent reports in the literature suggesting that repeated vaccination interferes with immunogenicity [23, 38, 34, 39-40]. Smith et al. [23] found that when vaccine components are unchanged or antigenically similar between strains of consecutive vaccines, the serologic response is diminished. The resulting magnitude remains unclear. In our study, B/Brisbane had a direct immune response with the corresponding 2010-11 IIV3 component of B/Brisbane. B/Malaysia was antigenically similar as another B/Victoria lineage virus and B/Florida was the most antigenically different from the vaccine strain being a B/Yamagata virus. These progressive divergences in antigenic similarity to vaccine components correspond to our resulting GMR responses with B/Brisbane seeing the greatest increase in GMR, followed by B/Malaysia and then B/Florida. For the four prior years of vaccination, three years also incorporated a B/Victoria lineage virus.

A possible explanation for effect modification by prior vaccination history is confounding by individuals with poor immune systems being more likely to receive vaccines than those who refuse vaccination [41]. Selection bias for a convenience sample is of concern but unlikely due to controlling for factors while estimating GMT. Although residual confounding is possible, we observed no significant associations between the A(H1N1)pdm09 monovalent vaccine in 2009 for any B virus preseason GMT or fold change. Since this vaccine contains no B component, we expected, and observed, no significant results implying the association between vaccination history and immune response is not driven by other unknown factors.

One of the limitations of our study is the questioned reliability of HI tests for influenza B viruses. A study by Monto et al. exhibited improved HI testing when treating the sample with ether [41]. Our serologic methodology did not include ether treatment, which may partly explain low GMT estimates. Low estimates due to the laboratory testing's inability to detect rising antibody titers limits the reliability of our results. However, despite these low overall estimates, we do have the capability to see significant differences between HCP with no prior vaccinations and HCP with 1-4 prior vaccinations. Reduced sensitivity due laboratory testing would also reduce differences seen in different vaccination groups. Since these differences are still apparent, there may be potentially greater differences in GMT estimates between the 0 prior vaccination group and 1-4 prior vaccination groups. We only used HI response to indicate immune response and could not test humoral or cell-mediated responses. Using microneutralization assays or B-cell response may provide additional insight into the mechanisms of the immune response.

Another limitation is the lack of information on past infections. Associations between vaccination history and immune response are likely impacted by exposure to natural infections and the corresponding immune response. If an individual is unvaccinated for the influenza season, this individual may have increased risk of exposure to wild influenza virus that may provide an even more comprehensive form of immunogenicity. Although we lack information on natural infections in the four previous seasons, this exposure is not exhibited in our preseason antibody titers. Those without prior vaccination were without known exposure and thus had a lower baseline compared to those with at least one exposure to vaccine. We also observed no effect modification by age in our study which would most likely occur since older age would provide more "opportunities" to experience natural influenza infection in their lifetime.

While our findings suggest a decrease in immunogenicity for influenza B with repeated vaccinations, annual vaccination remains one of the safest and most effective prevention strategy to protect HCP from infection and transmission of influenza [42, 43]. Developing sufficiently effective vaccines for each influenza season remains a challenge, demonstrated by sub-optimal vaccine effectiveness in recent years [43-46]. Future studies should prospectively address the limitations identified in our study to better delineate the role of repeated vaccinations in the protection against influenza B.

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Tables

Table **1**. Characteristics of 295 2010-11 Vaccinees and the Spearman Correlations Between These Factors and the Total Number of Inactivated Trivalent Influenza Vaccinations (IIV3s) during the Prior 4 Seasons.

	IIV3 Vaccinees [a]	**Number of Prior Vaccinations**
Categorical descriptors, N (%)		
Sex (Female)	249 (84)	.14 *
Race (White)	216 (73)	-.10
Ethnicity (Hispanic)	44 (15)	-.03
Child (age <13) at home	93 (32)	-.10
Physician	28 (9)	-.12 *
Work in Hospital	198 (67)	-.22 ***
Work in Emergency Dept.	70 (24)	-.09
Chronic medical condition [c]	155 (53)	.02
Continuous descriptors, Median (SD)		
Age (years)	47 (12)	.25 ***
Household size (0-7)	2 (1)	-.13 *
Education (5-levels)	3 (1)	.09
Self-rated health status (5-levels)	4 (1)	.02
Subjective Social Position (6-levels)	3 (1)	.03
Body mass index (kg/m^2)	28 (7)	.05
Direct patient care per week (hours)	35 (11)	-.01
Sleep Duration (hours)	7 (1)	.01
Sleep Latency (minutes)	15 (19)	.00
Sleep Quality (4-levels)	3 (0)	.07

* p < .05; ** p < .01; *** p < .001

[a] Sample (N = 295) includes health care personnel who received 2010-11 trivalent inactivated influenza vaccine (IIV3) and who had medical and vaccination records since July, 2006 and excludes those who received live attenuated influenza vaccine (LAIV) in 2010-11 and/or in any season since 2006-07.

[b] Fold-change is simply the ratio of pre-vaccination to post-vaccination titers.

[c] Presence of a chronic medical condition was identified by a medical visit during the prior year in the electronic medical record for a medical condition associated with increased risk of influenza complications.

Table **2**. Characteristics of 295 2010-11 Vaccinees and the Spearman Correlations Between These Factors and Preseason Geometric Mean Titer (GMT) for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria) and Post-Vaccination Fold-Change in Titers.

	Immune Response to 2010-11 Vaccine Component		Cross-Reactivity to 2010-11 Vaccine Component			
	B/Brisbane		B/Florida		B/Malaysia	
	Preseason GMT	**Post-Vaccination Fold Change** [a]	**Preseason GMT**	**Post-Vaccination Fold Change** [a]	**Preseason GMT**	**Post-Vaccination Fold Change** [a]
Categorical descriptors, N (%)						
Sex (Female)	.01	.06	.04	.06	-.01	.11
Race (White)	-.04	.12 *	-.04	.06	-.03	.07
Ethnicity (Hispanic)	-.09	.12 *	-.05	.03	-.06	.09
Child (age <13) at home	.01	-.02	-.05	-.07	.06	.01
Physician	-.05	-.04	-.06	-.04	.01	-.06
Work in Hospital	.05	.06	.02	.01	.06	.04
Work in Emergency Dept.	.03	.06	.01	.01	.02	.01
Chronic medical condition [b]	.10	.00	.00	-.07	.05	.02
Continuous descriptors, Median (SD)						
Age (years)	-.12 *	.05	-.11	.09	-.15 *	-.01
Household size (0-7)	.00	.07	-.09	-.06	.08	.03
Education (5-levels)	-.05	-.09	.01	-.07	-.04	-.09
Self-rated health status (5-levels)	.06	-.10	-.01	-.03	.06	-.12 *
Subjective Social Position (6-levels)	-.03	.09	-.06	.07	-.04	.05
Body mass index (kg/m^2)	.05	.16 **	-.01	.15 *	.04	.14 *
Direct patient care per week (hours)	.10	-.02	.05	.03	.07	.02
Sleep Duration (hours)	.02	.07	.02	.13 *	-.05	.11
Sleep Latency (minutes)	.07	-.07	.01	.01	.00	-.05
Sleep Quality (4-levels)	-.03	.07	.02	.09	-.02	.00

* $p < .05$; ** $p < .01$; *** $p < .001$

[a] Fold-change is simply the ratio of pre-vaccination to post-vaccination titers.

[b] Presence of a chronic medical condition was identified by a medical visit during the prior year in the electronic medical record for a medical condition associated with increased risk of influenza complications.

Table **3**. Characteristics of 295 2010-11 Vaccinees and the Spearman Correlations (with 95% CI) Between the Number of Inactivated Influenza Vaccinations (IIV3s) during the Prior Four Seasons and Preseason Geometric Mean Titer (GMT) and Post-Vaccination Fold Change in Titers for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria).

			Immune Response to 2010-11 Vaccine Component		**Cross-Reactivity to 2010-11 Vaccine Component**			
			B/Brisbane		**B/Florida**		**B/Malaysia**	
	IIV3 Vaccinees [a]	**Number of Prior Vaccinations**	**Preseason GMT**	**Post-Vaccination Fold Change** [b]	**Preseason GMT**	**Post-Vaccination Fold Change** [b]	**Preseason GMT**	**Post-Vaccination Fold Change** [b]
Prior vaccinations, N (%)								
2006-07 IIV3	169 (57)	.74 ***	.08 (-.03, .19)	-.19 ** (-.30, -.08)	.15 ** (.04, .26)	-.11 (-.22, .00)	.08 (-.03, .20)	-.23 *** (-.34, -.12)
2007-08 IIV3	144 (49)	.73 ***	-.02 (-.13, .10)	-.15 ** (-.26, -.04)	.11 * (.00, .23)	-.05 (-.16, .07)	.00 (-.12, .11)	-.15 ** (-.26, -.04)
2008-09 IIV3	180 (61)	.69 ***	.01 (-.12, .11)	-.14 * (-.25, -.02)	.08 (-.03, .20)	-.10 (-.21, .02)	.04 (-.08, .15)	-.14 * (-.25, -.02)
2009-10 IIV3	255 (86)	.45 ***	.14 * (.02, .25)	-.24 *** (-.34, -.13)	.06 (-.06, .17)	-.14 * (-.25, -.03)	.13 * (.01, .24)	-.22 *** (-.32, -.10)
2009 MIV A(H1N1)pdm09	129 (44)	.17 **	-.07 (.18, .05)	.04 (-.08, .15)	-.07 (-.18, .05)	.07 (-.04, .18)	-.08 (-.19, .03)	.00 (-.11, .11)
Sum of Prior IIV3, Mean (SD)	3 (1)	1 ***	.04 (-.08, .15)	-.23 *** (-.34, -.12)	.15 * (.03, .26)	-.13 * (-.24, -.01)	.06 (-.05, .18)	-.24 *** (-.35, -.13)

* p < .05; ** p < .01; *** p < .001

[a] Sample (N = 295) includes health care personnel who received 2010-11 trivalent inactivated influenza vaccine (IIV3) and who had medical and vaccination records since July, 2006 and excludes those who received live attenuated influenza vaccine (LAIV) in 2010-11 and/or in any season since 2006-07.

[b] Fold-change is simply the ratio of pre-vaccination to post-vaccination titers.

Note: Boxes encompass preseason GMT and post-vaccination fold change results when associations with specific prior IIV3 are expected to occur based on vaccine component

Table **4**. Serum Hemagglutination Inhibition Antibody (HI) Titers for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria) at Preseason and Post-Vaccination by Number of Inactivated Influenza Vaccinations (IIV3s) during the Prior Four Seasons, including Estimated Geometric Mean Titer (GMT) and Geometric Mean Fold Change Ratios (GMRs), among Healthcare Personnel Vaccinated with 2010-11 IIV3.

			Preseason (Time 1)	**Post-Vaccination (Time 2)**	
	Sum of IIV3 2006-07 to 2009-10	**N**	**GMT (95% CI)**	**GMT (95% CI)**	**GMR (95% CI)**
Immune Response to B/Brisbane	All	295	9.8 (9.6 - 10.1)	12.0 (11.7 - 12.3)	1.22 (1.20 - 1.24)
	4 IIV3s	79	10.1 (9.6 - 10.5)	11.1 (10.6 - 11.6)	1.10 (1.08 - 1.13)
	3 IIV3s	91	10.4 (10.0 - 10.8)	11.7 (11.3 - 12.2)	1.13 (1.11 - 1.16)
	2 IIV3s	52	10.0 (9.5 - 10.6)	11.5 (10.9 - 12.0)	1.14 (1.10 - 1.19)
	1 IIV3s	55	10.6 (10.0 - 11.2)	12.3 (11.8 - 12.8)	1.16 (1.11 - 1.21)
	0 IIV3s	18	8.4 (7.5 - 9.4)	13.5 (12.2 - 15.0)	1.61 (1.43 - 1.81)
Cross-Reactivity to B/Florida	All	295	10.7 (10.4 - 11.0)	11.6 (11.3 - 12.0)	1.09 (1.07 - 1.11)
	4 IIV3s	79	11.2 (10.7 - 11.7)	11.7 (11.2 - 12.2)	1.04 (1.01 - 1.08)
	3 IIV3s	91	11.3 (10.9 - 11.8)	11.9 (11.4 - 12.4)	1.05 (1.03 - 1.08)
	2 IIV3s	52	10.9 (10.2 - 11.7)	11.7 (11.0 - 12.3)	1.07 (1.03 - 1.10)
	1 IIV3s	55	10.4 (9.7 - 11.2)	11.3 (10.6 - 12.1)	1.09 (1.05 - 1.13)
	0 IIV3s	18	9.9 (8.7 - 11.2)	11.7 (10.5 - 13.0)	1.18 (1.10 - 1.28)
Cross-Reactivity to B/Malaysia	All	295	10.0 (9.8 - 10.3)	11.7 (11.5 - 12.0)	1.17 (1.15 - 1.19)
	4 IIV3s	79	10.3 (9.9 - 10.8)	11.2 (10.8 - 11.7)	1.09 (1.06 - 1.12)
	3 IIV3s	91	10.7 (10.3 - 11.1)	11.6 (11.2 - 12.1)	1.09 (1.07 - 1.11)
	2 IIV3s	52	10.1 (9.6 - 10.7)	11.4 (10.9 - 11.9)	1.13 (1.09 - 1.16)
	1 IIV3s	55	10.7 (10.2 - 11.2)	12.2 (11.6 - 12.7)	1.14 (1.10 - 1.18)
	0 IIV3s	18	8.8 (8.0 - 9.8)	12.5 (11.2 - 14.0)	1.42 (1.30 - 1.56)

Note: All estimates adjusted for sex, race, age (years) and age-squared, work in a hospital setting or not, household size, and education (years). Among vaccinees, GMR at time 2 describes change in GMT post-vaccination (or since Time 1); at Time 2. GMRs were calculated using the log-transformed HI titers. GMTs were converted back to original GMT values. The GMR estimate was calculated by 2 to the power of mean difference estimate.

Figures

Figure **1**. Preseason and post-vaccination GMT results for B/Brisbane immune response with 0-4 prior vaccinations among healthcare personnel.

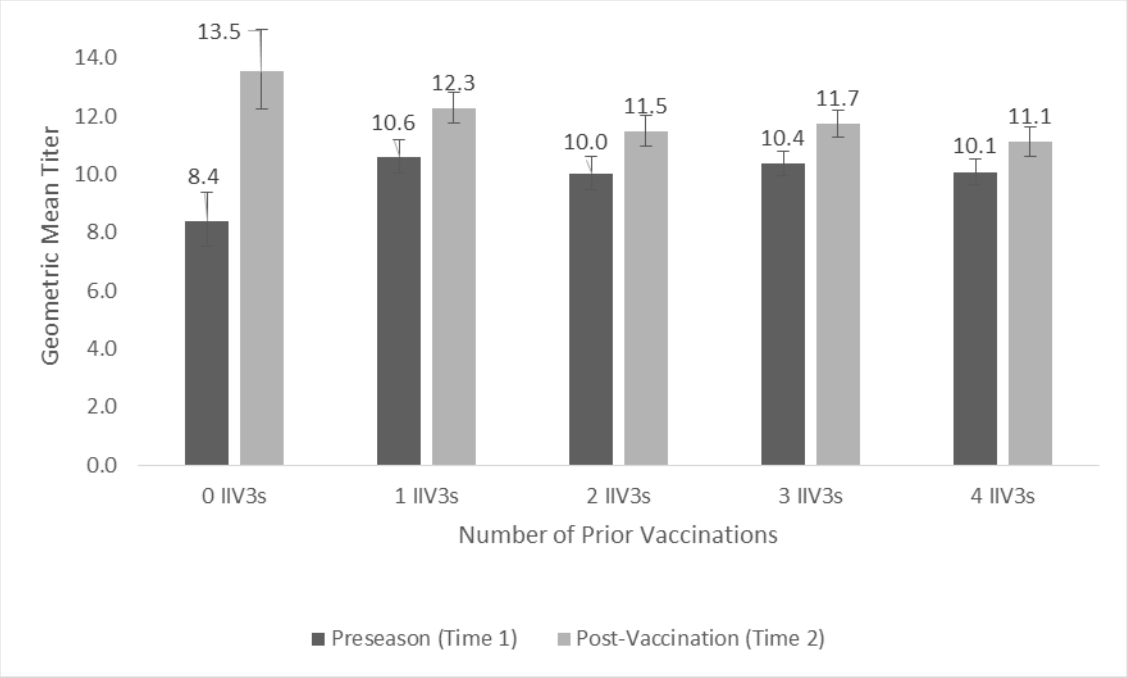


Figure **2**. Preseason and post-vaccination GMT results for B/Florida cross-reactivity with 0-4 prior vaccinations among healthcare personnel.

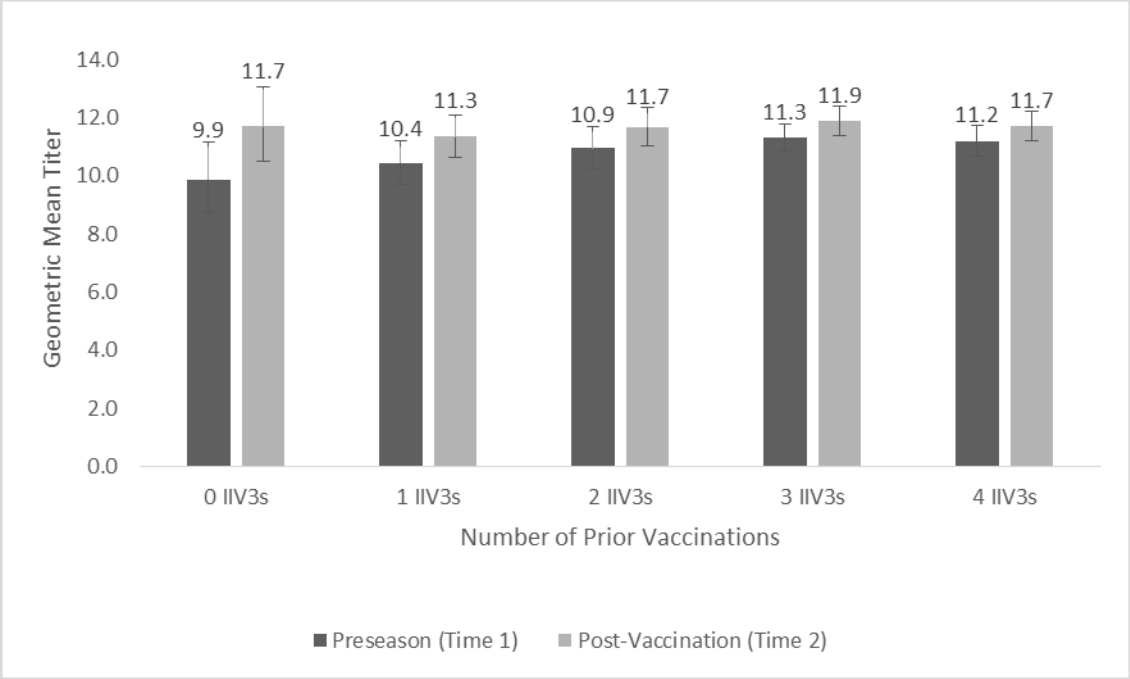


Figure **3**. Preseason and post-vaccination GMT results for B/Malaysia cross-reactivity with 0-4 prior vaccinations among healthcare personnel.

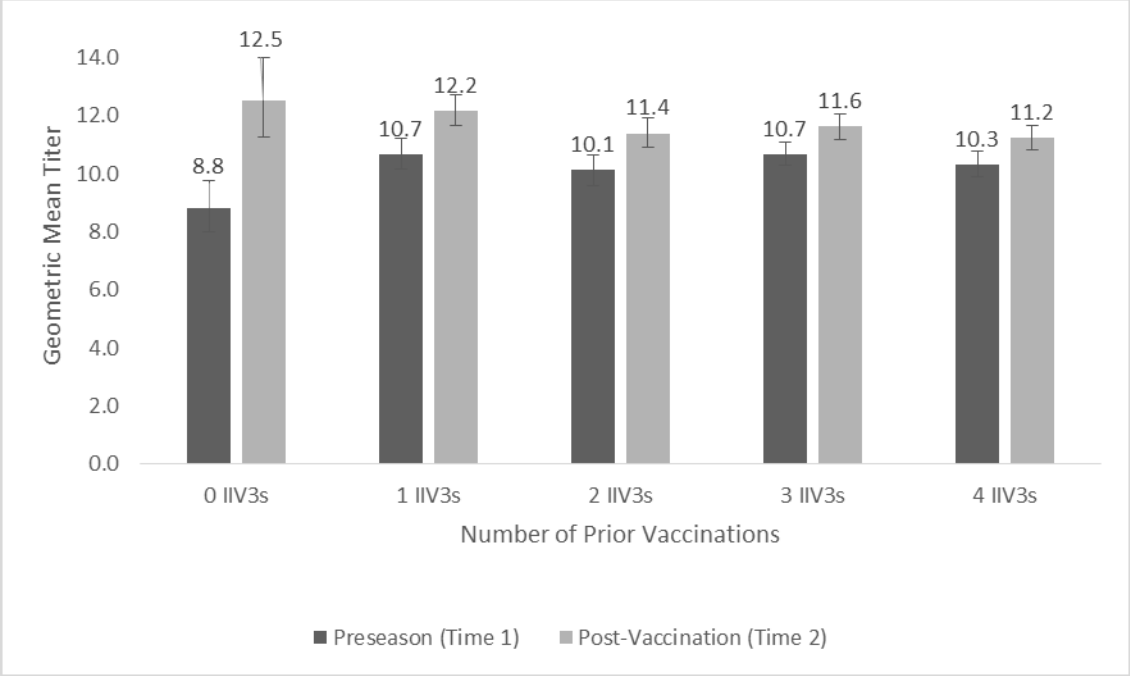
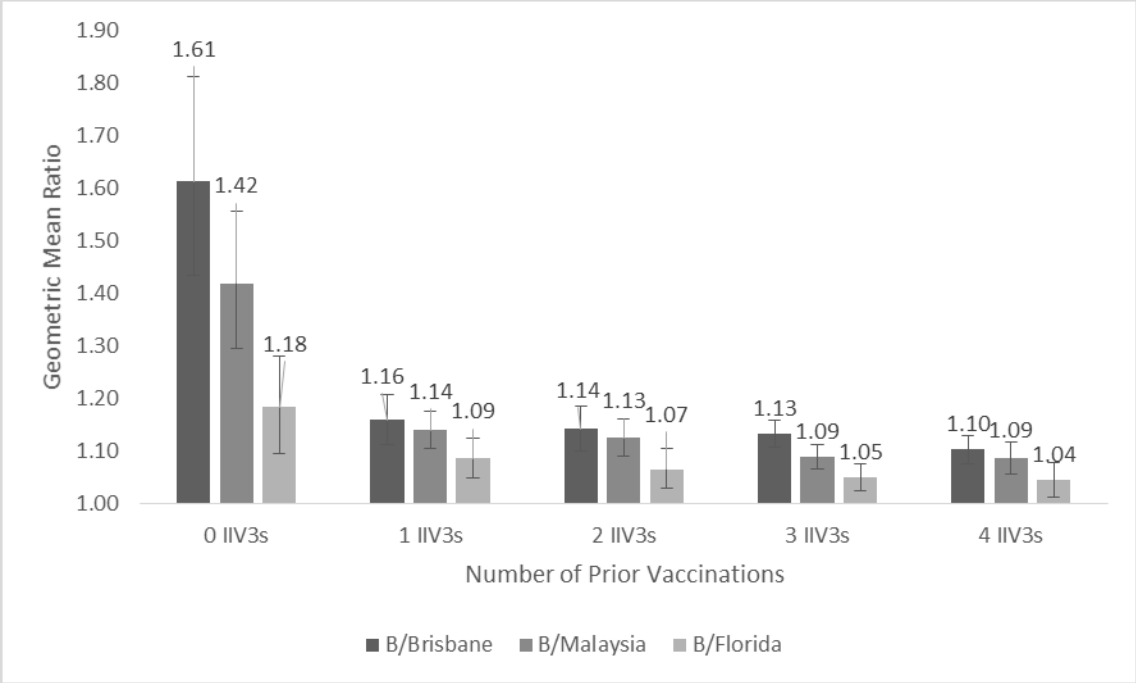


Figure **4**. Adjusted GMR for B/Brisbane immune response and B/Malaysia, B/Florida cross-reactivity for 0-4 prior vaccinations among healthcare personnel.



Chapter III: Extended Discussion

Public Health Implications

Routine vaccination is one of the most efficient and cost-effective method to prevent influenza infection, transmission, and illness. Vaccination is recommended for all persons, and in particular, those at great risk for illness such as pregnant women, immunocompromised, children, and older adults. Healthcare personnel are another important group in vaccination due to their direct work with the previously mentioned groups, but also in that they may be at more risk themselves for infection from ill patients. Vaccinated HCP may be less likely to transmit influenza to patients that may be at significant risk for adverse outcomes. While vaccination should be implemented to protect healthcare workers and patients, this study highlights the importance in understanding the interplay between immunogenicity and vaccination history.

Our results consistently display statistical similarity between preseason GMT among HCP with 1-4 prior vaccinations. Conversely, those with 0 prior vaccinations demonstrate lower preseason GMT than those with any vaccination. This implies that receiving just one vaccine may provide an increased baseline GMT for the subsequent season. Conversely, after receiving one vaccination, the association with increased baseline GMT does not occur with the subsequent season.

Conversely to preseason GMT, post-vaccination GMT tended to be the highest among HCP with no prior vaccinations. Those with 1-4 prior vaccinations maintained similar post-vaccination levels. This observation is important as it may imply that having no past exposure to influenza vaccination or infection induces the greatest immune response compared to those with repeated exposures.

GMR among HCP with no prior vaccinations are also higher than those with 1-4 prior vaccinations. The driving force behind this is the decrease preseason GMT levels among HCP with no prior vaccinations. In this aspect, these HCP had the greatest potential in immune response and cross-reactivity by starting with a lower floor. However, it is important to note that post-vaccination GMTs being slightly higher among those with no prior vaccinations compared to those with 1-4 prior vaccinations implies a more effective boost for higher post-vaccination titers.

Possible Future Directions

This study assessed immunogenicity from the 2010-11 IIV3 among HCP for three different B viruses: B/Brisbane/60/2008, B/Florida/4/2006, and B/Malaysia/2506/2004. The 2010-11 IIV3 was composed of a B/Brisbane component that directly matches B/Brisbane for the immune response. B/Florida and B/Malaysia were both analyzed for cross-reactivity. Despite the vaccine component not matching these two types of viruses, a degree of immunogenicity is still generated.

This degree of immunogenicity in cross-reactivity is still largely unknown. Antigenic similarity is what is typically seen as the driving force behind vaccine effectiveness across different B viruses [23]. This was seen explicitly in our data. Based on antigenic similarity, we would expect that B/Brisbane would have the greatest response to vaccine since it is a direct immune response. We would consider B/Malaysia to have the next greatest response to the vaccine, despite undergoing a cross-reactivity from the B/Brisbane component. The vaccine component and B/Malaysia are both under the B/Victoria lineage. B/Florida, on the other hand, is a B/Yamagata

lineage B virus. This would make B/Florida the least similar to the vaccine component and thusly produce the weakest response from vaccine.

Figure **4** shows a step-wise association between these three viruses in the predicted order. This trend was consistent among each vaccination history group but most profound among those with no prior vaccinations.

This interplay of cross-reactivity between vaccine components and different B viruses produces a complicated matrix when assessing vaccination history. Each IIV3 in the study produces some degree of immune response or cross-reactivity for each type of virus (Table **3**). Even when the vaccine component is not a match to that specific virus, significant associations in post-vaccination fold-change were not uncommon.

These vaccine component associations grow even more complicated when accounting for vaccine matching (Appendix Table **A**). For example, although the 2008-09 IIV3 component was B/Florida/4/2006, it was only a 17% match to circulating wild-type viruses that season. Since vaccine components are synthetically derived, they do not necessarily match the wild virus to promote protection from that wild virus. If the vaccine is simply produced ineffectively, the immunogenicity cannot be expected to be as strong as a well-matched vaccine. Further, while the 2008-09 vaccine may have been a poor match for B/Florida viruses, it may have an even better association with a different B virus due to the vaccine being antigenically closer to that type of B virus. In our data, we see that the 2008-09 IIV3 was not associated with post-vaccination fold change with B/Florida but was associated with B/Brisbane and B/Malaysia.

Appendices

Appendix Table A. Vaccine components for seasonal inactivated trivalent influenza vaccines (IIV3)

Seasonal Vaccines	Recommended Northern Hemisphere Vaccine Strains	Percentage of US CDC Tested Viruses Considered Vaccine Strain-like [a]
2010-11 IIV3	A/California/7/2009 (H1N1)-like virus A/Perth/16/2009 (H3N2)-like virus B/Brisbane/60/2008-like virus (B/Victoria)	94%
2009-10 IIV3	A/Brisbane/59/2007 (H1N1)-like virus A/Brisbane/10/2007 (H3N2)-like virus B/Brisbane/60/2008-like virus (B/Victoria)	100%
2008-09 IIV3	A/Brisbane/59/2007 (H1N1)-like virus A/Brisbane/10/2007 (H3N2)-like virus B/Florida/4/2006-like virus (B/Yamagata)	17%
2007-08 IIV3	A/Solomon Islands/3/2006 (H1N1)-like virus A/Wisconsin/67/2005 (H3N2)-like virus B/Malaysia/2506/2004-like virus (B/Victoria)	2%
2006-07 IIV3	A/New Caledonia/20/99 (H1N1)-like virus A/Wisconsin/67/2005 (H3N2)-like virus B/Malaysia/2506/2004-like virus (B/Victoria)	77%

[a] Past weekly surveillance reports are available on US CDC public website: http://www.cdc.gov/flu/weekly/pastreports.htm

Appendix Table B. Unadjusted Serum Hemagglutination Inhibition Antibody (HI) Titers for B/Brisbane/60/2008 (B/Victoria), B/Florida/4/2006 (B/Yamagata), and B/Malaysia/2506/2004 (B/Victoria) at Preseason and Post-Vaccination by Number of Inactivated Influenza Vaccinations (IIV3s) during the Prior Four Seasons, including Estimated Geometric Mean Titer (GMT) and Geometric Mean Fold Change Ratios (GMRs), among Healthcare Personnel Vaccinated with 2010-11 IIV3.

			Preseason (Time 1)	Post-Vaccination (Time 2)	
	Sum of IIV3 2006-07 to 2009-10	N	GMT (95% CI)	GMT (95% CI)	GMR (95% CI)
Immune Response to B/Brisbane	All	295	10.1 (9.9 - 10.4)	11.7 (11.5 - 12.0)	1.16 (1.13 - 1.18)
	4 IIV3s	79	10.1 (9.6 - 10.6)	11.1 (10.6 - 11.7)	1.10 (1.08 - 1.13)
	3 IIV3s	91	10.4 (9.9 - 10.8)	11.7 (11.3 - 12.2)	1.13 (1.10 - 1.16)
	2 IIV3s	52	10.0 (9.5 - 10.6)	11.5 (10.9 - 12.0)	1.14 (1.10 - 1.19)
	1 IIV3s	55	10.6 (10.0 - 11.2)	12.3 (11.8 - 12.8)	1.16 (1.11 - 1.20)
	0 IIV3s	18	8.4 (7.7 - 9.2)	13.5 (12.0 - 15.2)	1.61 (1.39 - 1.87)
Cross-Reactivity to B/Florida	All	295	11.0 (10.7 - 11.2)	11.7 (11.4 - 12.0)	1.07 (1.05 - 1.08)
	4 IIV3s	79	11.2 (10.7 - 11.8)	11.7 (11.2 - 12.3)	1.04 (1.01 - 1.08)
	3 IIV3s	91	11.3 (10.9 - 11.8)	11.9 (11.4 - 12.4)	1.05 (1.03 - 1.08)
	2 IIV3s	52	10.9 (10.2 - 11.7)	11.7 (11.0 - 12.4)	1.07 (1.03 - 1.10)
	1 IIV3s	55	10.4 (9.7 - 11.2)	11.3 (10.6 - 12.1)	1.09 (1.05 - 1.13)
	0 IIV3s	18	9.9 (8.8 - 11.1)	11.7 (10.4 - 13.2)	1.18 (1.07 - 1.31)
Cross-Reactivity to B/Malaysia	All	295	10.4 (10.1 - 10.6)	11.6 (11.4 - 11.9)	1.12 (1.10 - 1.14)
	4 IIV3s	79	10.3 (9.9 - 10.8)	11.2 (10.8 - 11.7)	1.09 (1.06 - 1.12)
	3 IIV3s	91	10.7 (10.3 - 11.1)	11.6 (11.2 - 12.0)	1.09 (1.07 - 1.11)
	2 IIV3s	52	10.1 (9.6 - 10.7)	11.4 (10.9 - 11.9)	1.13 (1.09 - 1.16)
	1 IIV3s	55	10.7 (10.1 - 11.2)	12.2 (11.6 - 12.7)	1.14 (1.10 - 1.18)
	0 IIV3s	18	8.8 (8.1 - 9.6)	12.5 (11.3 - 13.9)	1.42 (1.28 - 1.58)

Note: All estimates are unadjusted for potential confounding. Among vaccinees, GMR at time 2 describes change in GMT post-vaccination (or since Time 1); at Time 2. GMRs were calculated using the log-transformed HI titers. GMTs were converted back to original GMT values. The GMR estimate was calculated by 2 to the power of mean difference estimate.