

Evaluation of influenza vaccine effectiveness

*A guide to the design and interpretation of
observational studies*

Immunization, Vaccines and Biologicals



**World Health
Organization**

Evaluation of influenza vaccine effectiveness: a guide to the design and interpretation of observational studies

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Abbreviations & acronyms

ACIP	Advisory Committee on Immunization Practices of the United States of America
ARI	Acute respiratory infection
CDC	Centers for Disease Control and Prevention of the United States of America
COPD	Chronic obstructive pulmonary disease
EPI	Expanded Programme on Immunization
FluCAN	Influenza Complications Alert Network in Australia
GISRS	WHO Global Influenza Surveillance and Response System
HA	Influenza hemagglutinin glycoprotein
Hib	Haemophilus influenzae type b
IIV	Inactivated influenza vaccine
ILI	Influenza-like illness
I-MOVE	Influenza Monitoring of Vaccine Effectiveness (I-MOVE) network in Europe
ICU	Intensive care unit
IVR	WHO Initiative for Vaccine Research
NA	Influenza neuraminidase glycoprotein
PCV	Pneumococcal conjugate vaccine
RCT	Randomized clinical trial
REVELAC-I	Multicentre network for evaluation of influenza vaccine effectiveness in Latin America
RT-PCR	Reverse transcription-polymerase chain reaction
SARI	Severe acute respiratory infection
SPSN	Canadian Sentinel Practitioner Surveillance Network
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
VE	Vaccine effectiveness
WHO	World Health Organization

1. Introduction

1.1 Purpose of this guide and target audience

Influenza causes an estimated 444 000–553 000 deaths annually worldwide.¹ Most of these deaths occur among elderly adults and persons with chronic cardiopulmonary disease, but the toll includes 28 000–111 000 deaths among children less than five years of age.² In view of this global disease burden, many countries have implemented influenza vaccination programmes in order to reduce morbidity and mortality due to influenza. WHO recommends that countries consider influenza vaccination for persons at high risk of death or serious illness from influenza and has recognized the following groups to be at increased risk: pregnant women, children aged 6–59 months, elderly adults, individuals with specific chronic medical conditions, and health-care workers.³

Historically, influenza immunization programmes have been implemented mainly in high-income countries. In recent years, however, middle-income countries have initiated influenza immunization programmes, and policy-makers in low- and middle-income countries are increasingly assessing whether and how to implement new influenza immunization programmes. Policy-makers have faced similar decisions regarding introduction of other vaccines in recent years, including vaccines against rotavirus,⁴ *Haemophilus influenzae* type b (Hib), and *Streptococcus pneumoniae*.⁵ Decisions about introducing new vaccines are based on a thorough understanding of the burden of disease, the performance of the vaccines in preventing severe disease, the anticipated impact of a programme, national capacity, and other appropriate considerations. Efforts to expand introduction of rotavirus, Hib, and *S. pneumoniae* vaccines have led to well-established guidance for evaluating the effectiveness of vaccines and impact of vaccination programmes.⁶ However, several features of influenza epidemiology and vaccines create unique challenges for the evaluation of vaccine effectiveness (VE) and the expected benefits of influenza vaccination programmes. In this guide these challenges are described and the role of observational (non-randomized) influenza VE studies in evaluating influenza vaccination programmes is discussed. The designs for such studies are described and their advantages and limitations reviewed.

This guide is written primarily for researchers who design observational influenza VE studies and for public health scientists who interpret and apply the results of these studies. For researchers, it illustrates critical considerations in the design and analysis of influenza VE studies, as biased results may be produced even in settings where data completeness and quality are high. For public health scientists, it shows why VE studies have had a larger role in policy for influenza vaccines than for other vaccines.

The guide also addresses the critical evaluation of influenza VE studies by describing study limitations and errors that can lead to biased VE estimates and the contextual information that is necessary to properly interpret VE results.

Importantly, WHO does not recommend that VE studies be conducted by all countries with influenza vaccination programmes. VE studies are being conducted by a number of networks worldwide.⁷⁻⁹ The results of these existing studies can be expected to be applicable in other countries which have similar influenza epidemiology and similar vaccination programmes, in terms of vaccines used and risk groups targeted. The decision to carry out VE studies should be based on a need for country-specific VE estimates and on capacity to conduct rigorous VE studies.

1.2 Epidemiology of influenza

Influenza disease is primarily caused by influenza A and B viruses, which are spread from person to person by respiratory droplets and fomites.¹⁰ In temperate regions, both influenza A and B cause winter epidemics, with sporadic cases and outbreaks occurring out of season. In tropical regions, circulation of influenza viruses is more complex, ranging from seasonal peaks similar to those in temperate regions, to two or three annual incidence peaks, or to year-round virus circulation.¹¹

The influenza virus genes that encode the viral surface proteins undergo rapid genetic changes during viral replication. These accumulating genetic changes lead to changes in the conformation of surface proteins, a process known as antigenic drift.¹⁰ Antigenic drift results in reduced acquired immunity over time as antigenically novel viruses replace influenza viruses that previously circulated in the population. Influenza A viruses may also undergo a more dramatic change known as antigenic shift. In antigenic shift, virus strains emerge with novel surface antigens to which population immunity is low or absent. This is typically the result of reassortment of animal and human influenza A viruses. Such viruses have the potential to cause global pandemics.¹² Cumulative incidence of influenza virus infection during seasonal epidemics may reach 20–30% in children¹³ and 5–10% in adults¹⁴ and it has been estimated that influenza causes 444 000–553 000 deaths per year globally.¹ The risk of severe influenza may differ between high- and low-resource settings.²

Individuals at increased risk for complications of influenza virus infection include children <5 years of age, pregnant women, elderly adults, and individuals with chronic health conditions such as chronic heart or lung disease, asthma, and HIV/AIDS. Elderly adults have the highest rates of influenza-associated hospitalizations and deaths of any age group and may account for up to 90% of influenza-associated deaths from seasonal influenza viruses. Children aged <5 years, and particularly children aged <2 years, also have high rates of influenza-associated complications, with an estimated 1–2 million cases of influenza-associated severe acute lower respiratory infections and 28 000–111 500 deaths annually.² Pregnant women also have an increased risk of severe illness due to influenza.¹⁵

1.3 Influenza vaccines

Current trivalent influenza vaccines contain antigens of influenza A (H1N1), influenza A (H3N2) and one influenza B strain. Quadrivalent vaccines contain antigens of two influenza A strains and two B strains (Victoria and Yamagata lineages). The antigenic composition of the vaccines is adjusted each year. In an effort to match the antigens in the vaccine to those of the strains expected to circulate in the subsequent winter, the antigenic composition of the vaccines is reviewed prior to production for each hemisphere, with choice of formulation based on the characteristics of circulating influenza viruses tested within the WHO Global Influenza Surveillance and Response System (GISRS).¹⁶ Currently, influenza vaccines are produced at two separate times of the year, once for the northern hemisphere (with distribution generally beginning in September) and once for the southern hemisphere (with distribution generally beginning in March).¹⁷ Formulation may differ for each hemisphere or remain the same.

Many different influenza vaccine products are produced annually, by a number of different manufacturers. These include trivalent inactivated vaccines, quadrivalent inactivated vaccines, trivalent live attenuated vaccines, and quadrivalent live attenuated vaccines. Inactivated vaccines may be whole-virus vaccines, split virus vaccines (where the whole virus has been disrupted by detergent), or subunit vaccines (where the HA and NA components have been further purified from other virus antigens). Inactivated vaccines may also be produced in high-dose formulations or adjuvanted formulations. For any vaccine type, each manufacturer's formulation has its own safety profile and age group indications (which depend on country-level licensure and can change over time). VE is also expected to vary across vaccine products; for example, in elderly adults high-dose vaccines are expected to have greater VE than standard dose vaccines.¹⁸ At present, data are insufficient to assess whether VE varies across vaccine products within a given class, such as standard-dose non-adjuvanted inactivated vaccines. Pooled VE estimates across vaccine products suggest that influenza vaccines reduce the risk of medically attended influenza virus infection by approximately 50%, although there is considerable heterogeneity by season, setting, and population subgroup.^{8,19-21}

Influenza viruses vary in transmissibility and virulence from season to season and cause a wide range of adverse health outcomes. Influenza vaccines vary in degree of antigenic match to circulating viruses, and influenza VE can differ by age group, virus type/subtype, vaccine product, health status of vaccinees, health outcomes under study and the degree of antigenic match between vaccine and circulating viruses.²² VE can also differ between groups that have residual immunity to circulating viruses (from past exposure or prior vaccination) and those naive to circulating viruses. Public health scientists and others who support influenza VE studies need to be aware of the variability that is possible in influenza VE estimates. This requires careful consideration of study endpoints prior to initiating VE studies, and proper generalization of VE estimates in relation to study participant profiles, types/subtypes of contributing viruses, study location, and years when studies are completed.

1.4 Organization of this guide

The guide is organized in the following sections:

Section 2 describes the role of influenza VE studies in implementing and evaluating influenza vaccination programmes.

Sections 3–5 cover outcomes of interest, measuring vaccination history, and measuring potential confounders – topics of importance for any influenza VE study.

Section 6 reviews different designs that could be used for influenza VE studies, and the strengths and limitations of each.

Section 7 reviews statistical considerations for designing VE studies and for analysing VE study data.

Section 8 provides a more detailed description of one specific design that is most likely to be attempted in low-resource settings where influenza vaccines have been introduced to at least some parts of the population: a test-negative study built on an existing severe acute respiratory infection (SARI) surveillance system.

Section 9 describes key elements that need to be included in any report of an influenza VE study, so that the results can be properly interpreted.

2. The role of VE studies in influenza vaccination programmes

Two scientific considerations help determine whether to introduce a particular vaccine in a specific population. The first consideration is the burden of disease that is potentially preventable by vaccination. This disease burden can be assessed by disease surveillance systems²³ or by re-analysis of randomized clinical trial (RCT) data from vaccine efficacy studies as vaccine probe studies for selected clinical outcomes.^{24,25} The second consideration is the expected performance of the vaccine in practice. This can be assessed through RCTs of vaccine efficacy and through observational VE studies. Observational VE studies in countries with existing vaccine programmes can provide useful evidence for public health policy-makers in evaluating the expected VE in real-world conditions.

Vaccine Efficacy: Reduced risk of disease among vaccinated persons resulting from vaccination in ideal circumstances; estimated from randomized trials.

Vaccine Effectiveness: Reduced risk of disease among vaccinated persons attributed to vaccination in real-world conditions; estimated from observational (non-randomized) studies.

Vaccine Impact: Reduction in incidence of disease in a population where some members are vaccinated; usually estimated from ecologic (or modeling) studies. Vaccine impact results from direct effects of vaccination in the vaccinated, as well as indirect effects in the unvaccinated which are presumed to be due to herd immunity.

After a vaccine has been introduced in a population, additional studies help in evaluating the impact of the immunization programme. Data from VE studies can be used to inform vaccination policy details, such as determining whether the vaccine is effective in groups at high risk of severe disease or identifying preferred vaccine product classes. For example, observational influenza VE studies in the United States during the 2013/14, 2014/15, and 2015/16 influenza seasons led the ACIP to recommend against the use of live attenuated influenza vaccines for the 2016/17 influenza season.²⁶

For the purposes of this guide, “impact” refers to reduction in incidence of disease due to vaccination. The impact of vaccination programmes on disease burden is typically evaluated using active surveillance programmes that compare the incidence of disease prior to and after programme implementation.⁶ For certain vaccines, these assessments can include both the incidence of laboratory-confirmed outcomes, such as invasive *S. pneumoniae* disease, and of non-specific outcomes, such as hospitalizations for pneumonia, with the difference in incidence attributed to the vaccination programme. These so-called “before-after studies” applying interrupted time-series methods to surveillance data can show the degree to which introduction of the vaccine has reduced disease incidence. While they have been used for other vaccines, including

pneumococcal, Haemophilus influenzae type B (Hib), and rotavirus vaccines,^{6,27,28} they are difficult to conduct for influenza vaccines, necessitating alternative approaches.

2.1 Challenges to studying the impact of influenza vaccination programmes

In contrast to evaluation of other vaccine programmes, evaluating the impact of influenza vaccination programmes after vaccine introduction generally relies on repeated influenza VE studies. However, before-after studies to demonstrate vaccine impact are very difficult to conduct for influenza, for the following reasons:

- i) Influenza VE is modest compared to the effectiveness of other vaccines such as pneumococcal conjugate vaccine (PCV). RCTs suggest that a primary series of PCV in infancy reduces the risk of invasive pneumococcal disease due to vaccine serotypes by 70–95%, with protection lasting for several years.^{29,30} In contrast, influenza VE against laboratory-confirmed influenza virus infection is rarely higher than 60% and may sometimes be 30% or less, with protection that may wane from one season to the next or within a season.^{20,31–34} Even with high vaccine coverage, the expected decline in severe influenza disease will be substantially less than the decline in pneumococcal disease after implementation of a vaccination programme, making the impact of influenza vaccine more difficult to measure. Observing population-level impact is further complicated by the fact that influenza vaccine coverage is rarely high even in high-resource settings.^{35,36} For example, in the United States during 2015/16, where influenza vaccination was recommended for all persons ≥ 6 months of age, vaccine coverage was 46% in age-eligible persons, 59% in children aged ≥ 6 months, and 42% in adults.³⁷
- ii) Seasonal influenza epidemics display considerable heterogeneity, driven by population immunity and by antigenic drift of influenza viruses. This heterogeneity can affect the timing, incidence, and severity of epidemics; it also causes variations in the degree of similarity between antigens in the vaccine viruses and in the circulating viruses, also known as antigenic match. VE can vary from year to year depending on factors such as the degree of antigenic match between the influenza strains in the vaccine and the strains that circulate.³¹ Because of this heterogeneity, many years of surveillance both pre- and post-implementation would be needed to properly evaluate the impact of an influenza vaccination programme on disease incidence. If too few years are included in either the pre-implementation or the post-implementation periods, the conclusions could be biased. For example, if the pre-implementation period consisted of two years, both of which were relatively mild influenza seasons with A(H1N1) or B viruses predominating, the average burden of disease pre-implementation would be underestimated, making it difficult to demonstrate a decline in incidence post-implementation. Such studies have not been attempted even in high-resource settings with moderate vaccine coverage.

Beyond the impact of vaccination on burden of illness, policy-makers may wish to evaluate the economic impact of vaccination programmes. Economic impact is

estimated based on rates of outcomes averted at the population level and on estimates of cost of care. WHO is currently developing guidance on estimation of the economic impact of influenza vaccination programmes.³⁸

2.2 Use of VE studies to estimate impact of influenza vaccination programmes

Because of the heterogeneity in influenza seasons and the modest effectiveness of influenza vaccines, interrupted time series studies are unlikely to be feasible for assessing the impact of influenza vaccination programmes. Instead, influenza vaccination policy-makers typically have to combine data from multiple sources to estimate vaccine impact. The first step is to use influenza surveillance data to estimate the disease burden in terms of influenza-attributable hospitalizations, deaths, and / or (occasionally) outpatient clinic visits.³⁹ For example, a hospital-based surveillance system could attempt to enrol all patients meeting specified clinical criteria and test those patients for influenza. Collecting these surveillance data over multiple years would allow policy-makers to estimate the incidence of hospitalizations caused by influenza. The second step is to estimate influenza vaccine coverage among groups targeted for vaccination. The final step is to estimate influenza VE, which is often estimated on an annual basis due to the year-to-year variation in VE, particularly in settings with well-defined influenza seasonality.

Estimates of disease burden, vaccine coverage, and VE are then combined to provide estimates of the cases, hospitalizations, and deaths averted by vaccination. Among those vaccinated against influenza, the cases averted per 1000 vaccinees can be estimated as follows:

$$\text{Cases averted} = \frac{\frac{I}{1 - VE * p} - I}{p}$$

Where I is the cumulative incidence of influenza events per 1000 population, p is the vaccine coverage, and VE is the vaccine effectiveness. If vaccine coverage data are available by month, then more complicated modelling approaches are also possible.⁴⁰

Influenza VE studies have several important uses. Initial VE studies after the first introduction of influenza vaccine can verify that the vaccine works as predicted. Then ongoing VE studies are used as one input in models of outcomes averted by vaccination. To this end, many countries and regions have established platforms for ongoing annual estimates of influenza vaccine effectiveness, such as the European I-MOVE consortium,⁸ US Flu VE Network,²⁰ the Canadian Sentinel Practitioner Surveillance Network (SPSN),²¹ the Pan American Health Organization's REVELAC-I network,⁷ and the Australian FluCAN vaccine effectiveness surveillance.^{41,42} The VE estimates from these platforms are used to estimate the health impact of vaccination.^{43,44} Given the lack of a robust correlate of protection, the European Medicines Agency (EMA) has recently begun requesting observational studies as part of the manufacturer's Risk Management Plan.⁴⁵ The I-MOVE network and the European Centre for Disease Prevention and Control (ECDC) have useful websites with generic protocols, research publications, and reviews.⁴⁶⁻⁴⁹

3. Outcomes of interest for influenza VE studies

A number of outcomes have been used for influenza VE studies, reflecting the range of morbidity and mortality that influenza virus infection can cause and which may be preventable by vaccination. This section reviews several of these outcomes and evaluates the benefits and limitations of the use of each. As with other vaccine preventable diseases, such as pneumococcal pneumonia, selecting appropriate VE outcomes involves a trade-off between providing information that is of greatest interest to policy-makers and identifying outcomes that can be assessed with a minimum of misclassification or other type of bias. For the purposes of VE studies, outcomes can be classified according to two major considerations: the specificity for influenza virus infection, and the severity of the illness.

Outcome: A specific, measurable disease or health event. All study subjects should be at risk for the outcome, and the occurrence or non-occurrence of the outcome should be known for all subjects.

Outcome misclassification: Incorrectly determining the occurrence of the outcome for a study subject. This includes falsely considering a diseased person to be disease-free, and falsely considering a disease-free person to be diseased.

Study participants: All persons enrolled in a VE study. Outcomes, vaccine history, and covariates should be measured on all study participants.

- i) With respect to specificity, influenza vaccine is only effective against influenza virus and not against diseases that are not directly or indirectly caused by influenza virus infection. Laboratory-confirmed influenza illness is a highly specific influenza outcome. Even during the peak of the influenza season, the non-specific clinical syndrome of “influenza-like illness” (ILI) includes various other respiratory pathogens that cause a similar syndrome against which influenza vaccine does not protect.⁵⁰ The proportion of ILI not due to influenza varies by intensity of influenza and other respiratory virus activity, so while VE against ILI will always be lower than VE measured against laboratory-confirmed influenza outcomes, the extent of under-estimation will vary.
- ii) Influenza outcomes can be classified by severity, ranging from mild illnesses managed at home without any intervention or attended on an outpatient basis, to more severe outcomes of hospitalization, ICU admission or death.

The non-specific clinical presentation of influenza infection and the broad range of potential influenza complications create challenges for determining appropriate outcomes for influenza VE studies, as described below.

- i) Influenza virus infection can cause severe illness and death through a number of different pathways and clinical syndromes. Infected individuals can have severe influenza virus infections, generally characterized by cough and difficulty in breathing, reflecting lower respiratory involvement.^{51,52} Influenza can also cause severe illness by exacerbating existing chronic disease such as asthma or chronic obstructive pulmonary disease (COPD). For these patients, typical influenza disease signs such as fever may be absent. Furthermore, influenza virus infection can predispose individuals to secondary bacterial pneumonia. In such cases, the time from initial influenza virus infection to secondary pneumonia to hospitalization may be long enough that patients no longer shed detectable influenza virus when they present for medical care. Influenza virus infection may also trigger cardiopulmonary complications including acute myocardial infarction; patients presenting with these conditions are unlikely to be tested for influenza virus infection.⁵³ Due to the variety of potential complications of influenza virus infections, it is difficult to select outcomes for VE studies that capture the range of influenza-associated complications. Selecting only one outcome for a VE study (such as pneumonia) may underestimate the vaccine benefits because other important outcomes that influenza vaccine may prevent, such as exacerbation of COPD and cardiovascular disease, are overlooked.

- ii) Most of the clinical syndromes that influenza virus infection may cause can also be caused by other factors, including other respiratory pathogens. Severe respiratory disease may be caused by influenza, respiratory syncytial virus, and adenovirus, among others.⁵⁴ For any given clinical syndrome, such as severe acute respiratory infection (SARI) or ILI, the contribution of influenza to the total burden may range from moderate to low and will differ from year to year and between different locales. For example, the contribution of influenza to hospitalized pneumonia cases may range from <1% to 12% of such cases.⁵⁵ Because the majority of cases of these clinical syndromes are caused by pathogens other than influenza virus, an influenza vaccination programme will not lead to major reductions in the incidence of syndromes with multiple causes, such as pneumonia, or use of primary or secondary health-care services, such as seasonal increases in emergency department presentation. For example, assuming that influenza causes 10% of hospitalized pneumonia cases in a given age group and that influenza VE against influenza is 50%, the expected VE against the syndrome of pneumonia would be only 5% (50% prevention of 10% of pneumonia cases).⁵⁶ A vaccine with a low VE against a serious and relatively common clinical syndrome might be of great public health value. However, detecting a low VE requires a very large sample size and a correspondingly large research investment, as well as reasonable uptake of vaccine in the target population.

The following are some clinical outcomes that could be used as endpoints for influenza VE studies:

1. Non-specific (i.e. syndromic) outcomes

A. Severe acute respiratory infection

The WHO Global Epidemiologic Surveillance Standards for Influenza define SARI as an acute respiratory infection with history of fever or documented fever of ≥ 38 °C, cough, onset within the last ten days, and requiring hospitalization.³⁹ SARI offers several advantages as an outcome for influenza VE studies. Patients with SARI are by definition hospitalized and typically have severe illness. Depending on the stated programme objectives, this outcome is likely to be of particular interest to policy-makers, since severe illnesses are much more important in terms of financial and health costs (e.g. disability-adjusted life years) than mild illnesses. As many countries have existing systems of SARI surveillance, it may not be necessary to set up new surveillance systems to identify SARI cases for influenza VE studies, reducing the potential cost of VE studies.

SARI also has important limitations as an endpoint for observational influenza VE studies. Foremost is the fact that influenza makes at most only a modest contribution to the overall incidence of SARI. A two-year study in the Philippines, for example, found that only 9% of SARI cases had laboratory-confirmed influenza virus infection.⁵⁷ Even if influenza vaccine were 100% effective against laboratory confirmed influenza, an influenza VE study using SARI as an endpoint would find 9% effectiveness at most in this setting. An observational study would require a large sample size (and likely many study sites) to measure effectiveness with adequate precision. Another limitation is that, in some settings, influenza vaccine may be used disproportionately by persons with a different risk for SARI than unvaccinated persons (e.g. those with chronic heart disease). Differential vaccine utilization can cause very strong confounding of VE estimates in observational VE studies.⁵⁸

B. All-cause pneumonia requiring hospitalization

All-cause pneumonia requiring hospitalization has many of the same benefits and limitations as SARI as an endpoint for influenza VE studies. VE against severe outcomes is of high interest to policy-makers, and some countries have existing surveillance systems for hospitalized pneumonia cases, particularly among children <5 years of age. Pneumonia has the additional advantage of being a clinical diagnosis with which clinicians are familiar. However, the contribution of influenza to pneumonia is low, and observable VE against pneumonia will also be low. Furthermore, many influenza-associated pneumonia cases occur in adults aged at least 50 years, cases which are often not captured by existing pneumonia surveillance systems focused on children. Thus, using hospitalized pneumonia as an outcome will likely require study-specific surveillance systems to be developed. Pneumonia is also subject to confounding in the same way as is SARI.

C. Influenza-like illness

ILI is defined by WHO as an acute respiratory infection with measured fever of ≥ 38 °C, cough, and onset within the last seven days.³⁹ This definition was created primarily for the purpose of conducting virologic surveillance, i.e. for identifying the occurrence of influenza and collecting specimens which were likely to test positive for influenza virus. ILI as a diagnosis is typically applied to patients in outpatient settings. Outpatient ILI has several advantages for VE studies. It is relatively easy to apply the ILI case definition in setting up outpatient surveillance. At least in regions with well-defined influenza epidemics, the proportion of patients (during the epidemic period) who have influenza is higher among patients with ILI than among patients with SARI or pneumonia. This greater specificity of the outcome leads to larger VE estimates relative to SARI or pneumonia, which reduces the relative sample size needed to detect statistically significant VE.

ILI as a VE endpoint has several disadvantages. The main limitation is that illness treated on an outpatient basis is less compelling to policy-makers in developing countries, for whom influenza vaccine programmes are likely to be justified based on the expected impact on serious outcomes. While demonstrating VE against ILI would indicate that the vaccine can reduce the risk of influenza-associated outpatient illness, VE against ILI cannot be extrapolated to VE against more serious outcomes without making assumptions that are difficult to validate.

D. All-cause mortality

All-cause mortality was formerly a common endpoint for observational influenza VE studies, particularly among older adults.^{59,60} More recent studies from the United States have shown that among older adults, those at higher risk for death are less likely to receive influenza vaccine than those at lower risk for death, and that these differences in risk lead to strong confounding in VE estimates, often called the “healthy vaccinee bias”.^{61,62} It is very difficult to accurately estimate VE in view of this confounding, even when using sophisticated statistical methods and extensive medical record data.⁶³ All-cause mortality is not recommended as an endpoint for VE studies.

E. Adverse birth outcomes

In recent years, increasing attention has been paid to the possible benefits of maternal influenza vaccination on birth outcomes such as small for gestational age and low birth weight. Influenza virus infection in pregnant women may sometimes result in preterm birth, although the evidence for this is not consistent. If maternal influenza virus infection can cause poor birth outcomes, then influenza vaccination may prevent some of these adverse outcomes. Maternal influenza vaccination can also protect neonates and young infants from influenza virus infection.

A reduction in adverse birth outcomes or infant influenza virus infections would be an important public health benefit of influenza immunization programmes. However, WHO recommends caution with the use of adverse birth outcomes as endpoints of observational influenza VE studies, for several reasons:

- i) A major methodologic challenge in the study of adverse birth outcomes is the difficulty of accounting for the interrelated effects of calendar time and gestational age. Both the receipt of influenza vaccination and the periods of risk for influenza virus infection depend on calendar time (on the availability of vaccine and on the circulation of influenza viruses, respectively), and the timing of vaccination relative to gestational age depends jointly on the availability of vaccine and on the date of conception. Failure to properly account for these joint effects can bias VE estimates.
- ii) Confounding due to differences in risk of adverse outcomes between vaccinated and unvaccinated women is a strong possibility. Women at increased risk of preterm birth or other outcomes may be preferentially offered or seek vaccination. Unless study staff have access to relevant medical records or other data for identifying women at higher risk of adverse outcomes, this confounding will be very difficult to control.
- iii) A review of observational study design methodologies for studies assessing influenza vaccine association with adverse birth outcomes provides further rationale for recommending caution with the conduct and interpretation of these studies.^{64,65}

For any non-specific outcomes, assessment of influenza VE should be restricted to time periods when influenza is circulating in the source population. Enrolling subjects only during periods when influenza circulates will maximize the proportion of non-specific outcomes that are caused by influenza, which will in turn increase the ability of the study to detect any vaccine effects. Caution must be used in reporting these results, however, so that it is clear that the estimated VE is against the outcome during periods of influenza circulation only (i.e. when the outcome is more likely to be true influenza), not against the outcome year-round (i.e. when other causes of the non-specific outcome are more contributory).

2. Laboratory-confirmed outcomes

RT-PCR is the standard test for laboratory confirmation of influenza virus infection during acute illness. RT-PCR and other commercially available molecular diagnostic tests are highly sensitive and highly specific for detecting influenza viruses. Although other assays are available, including viral culture, rapid antigen tests, and immunofluorescence, these are less sensitive than RT-

PCR. Rapid antigen tests are also considerably less specific than RT-PCR and can cause substantial misclassification, as imperfect specificity is more likely to bias VE estimates than imperfect sensitivity.^{22,66} Rapid antigen tests have the further disadvantage of being unable to distinguish between influenza A subtypes or (for some tests) between influenza types A and B. Beyond laboratory confirmation of the presence of influenza virus infection, detection of the specific influenza type/subtype by RT-PCR allows researchers to stratify influenza VE estimates by virus type/subtype, as discussed in more detail in Section 8.

In general, laboratory-confirmed outcomes have several advantages over non-specific outcomes for influenza VE studies. Laboratory-confirmed outcomes are much more specific for influenza virus infection than are outcomes based on clinical signs and symptoms. Thus, VE estimates will be higher (and it will be easier to detect significant VE) when using laboratory-confirmed outcomes.

A major constraint with the use of laboratory-confirmed outcomes in influenza VE studies is the need for appropriate laboratory capacity, including specimen collection, handling, and storage as well as molecular assay technology and reagents. All of these activities require training of clinical staff to ensure standardized specimen collection and handling. Training of laboratory staff is also required to ensure proper processing and testing of specimens. This is particularly critical with the use of RT-PCR technology, which can give false positive results if specimens become contaminated during processing. Improper specimen handling or collection of inappropriate specimens can lead to false negative results. Furthermore, due to the need to enrol patients who will likely have detectable influenza virus, laboratory-confirmed outcomes are typically restricted to patients who present for illness within seven days of illness onset. This excludes patients who may have developed severe disease, such as secondary bacterial pneumonia, more than seven days after illness onset.

The decision to assess potential study subjects for laboratory-confirmed influenza virus infection should not be based on clinicians' decisions but on pre-specified protocol guidelines. Clinical decisions to test specimens for influenza can introduce bias into VE studies in a number of ways. Most obviously, when clinicians have access to vaccination records they may be less likely to order influenza testing in vaccinated patients, reasoning that vaccinated patients are less likely to have influenza than unvaccinated patients. This would falsely lower the number of vaccinated cases among the study participants and bias VE estimates upwards. Even in the absence of vaccination data, clinical testing decisions may introduce bias in more subtle ways. Among patients with acute respiratory illness (ARI), US physicians preferentially prescribe influenza antivirals to patients who are later found to test positive for influenza.⁶⁷ This suggests that physicians have some ability to distinguish influenza ARI from ARI due to other causes. Physicians may preferentially order testing of patients they suspect of having influenza (to validate their suspicion), or testing of patients whose influenza

status they are unsure of (to address their uncertainty). Either case may cause systematic misclassification of the potential pool of study subjects. To avoid these biases, study protocols should specify the symptoms, duration of illness, and other eligibility criteria for attempting to enrol and test patients for influenza. These criteria should then be applied to all (or to a random sample of) eligible patients regardless of clinical testing preferences.

Laboratory-confirmed outcomes, including both outpatient outcomes²⁰ and hospitalized outcomes,⁶⁸ are becoming standard for VE studies in high-resource countries. WHO recommends the use of laboratory-confirmed outcomes whenever possible because of the benefits associated with use of outcomes specific for influenza.

Recommended background research on outcomes to inform VE studies:

- 1) If non-specific outcomes such as SARI hospitalizations, pneumonia, or ILI are to be used, background research should estimate the incidence of these outcomes and the expected proportion that are caused by influenza. These data will be needed for sample size calculations to determine the feasibility of a VE study. They will also allow researchers to estimate the range of VE estimates that would be expected from a VE study. For example, if 20% of pneumonia hospitalizations were attributed to influenza in some target populations, VE against pneumonia is expected to be 20% or less, and should be around 10% if VE against influenza is 50%.
- 2) If laboratory-confirmed outcomes are to be used, researchers need an understanding of the testing process. If there is to be active testing of specimens collected from patients meeting a certain case definition, the study team needs to know how to systematically identify those patients. If the study will rely on clinical testing, the study team needs to evaluate the clinical decision process to order influenza tests, and particularly what patient characteristics are associated with clinical testing.
- 3) For cohort studies, the researchers need to determine the care-seeking patterns of the proposed study cohort. What facility/facilities will be used for outpatient care? Where will cohort members be hospitalized for severe illness? What proportion of outcomes may be missed due to seeking care outside of study facilities or self-treatment at home?
- 4) For traditional case-control studies, researchers need to understand the catchment areas of the study enrolment facilities. What is the source population for the study hospitals or clinics? Where else might ill persons in this population seek medical care? Who are the influenza vaccine providers for this population? This information will be needed to identify appropriate control groups.

4. Assessing influenza vaccination history

As part of an observational influenza VE study, each subject's influenza vaccination history must be ascertained. The ultimate goal is to assess whether the subject has received the current season's influenza vaccine prior to any outcomes of interest. This means that both the fact of vaccination and the approximate timing of vaccination need to be determined. Fact of vaccination must distinguish receipt of the currently recommended influenza vaccine from influenza vaccination in previous influenza seasons. For most influenza vaccines, it is recommended that children aged <9 years

who are vaccine-naïve should receive two doses separated over time when they are vaccinated for the first time. For these children, it is desirable to document previous influenza vaccinations to determine whether they are fully or partially vaccinated. In settings where multiple vaccine products may be in use, the type of vaccine product (e.g. inactivated, live, adjuvanted, high-dose) used should be documented if possible, ideally specifying actual product name.

Current influenza vaccination: Receipt of one or more doses of the current year's influenza vaccine.

Prior influenza vaccination: Receipt of one or more doses of influenza vaccine in previous years.

Vaccination misclassification: Falsely classifying a vaccinated subject as unvaccinated, or falsely classifying an unvaccinated subject as vaccinated, or falsely classifying person-time prior to vaccination as being post-vaccination.

Timing of vaccination is important to make sure subjects are counted as vaccinated only after they have received vaccine and there has been sufficient time since vaccination for an immune response to develop (generally assumed to be 14 days post-vaccination). This is particularly important if vaccination is being carried out during (rather than prior to) the period of peak influenza virus circulation.

Vaccination history can be assessed in several ways:

- i) One approach is to rely on administrative records created as part of vaccination programmes, such as patients' vaccination cards. Many vaccination programmes in low-resource settings focus on vaccines given to children <5 years of age, such as measles, mumps, and rubella vaccine and diphtheria, tetanus, and pertussis vaccine. Most countries have some established infrastructure for administering these vaccines and for keeping vaccination records, such as vaccination cards kept by parents, although influenza vaccine receipt is often not included in vaccination cards in many low-income countries. Many of the persons at high risk for influenza-associated complications are adults. Vaccination cards are not

usually given to adults, and without an established infrastructure, influenza vaccination may not be routinely recorded in clinic records, requiring reliance on self-reporting.

- ii) Another approach is to assess influenza vaccination status through national or provincial vaccination registries, either electronic immunization registries or paper EPI registries.⁶⁹ Where available, these registries can greatly facilitate the determination of the vaccination status of study subjects. However, registries are not perfect. In some settings it may be difficult to match a study subject to a record in the registry and data entry errors can lead to incorrect information in the registries. Even in high-income countries, data in vaccine registries are generally only complete for registry items that are required for entry of a record into the database (such as patient ID and vaccine name); items that are optional generally have substantial missing data, and it is often these fields that are of interest to researchers.⁷⁰ Registry recorded vaccine receipt has high specificity but variable sensitivity depending on quality controls.
- iii) Perhaps the most straightforward approach to assessing vaccination history is to ask subjects whether and when they (or their children, for parents of enrolled children) received the current year's influenza vaccine. In some settings, adult subjects have been able to accurately recall their influenza vaccination status, relative to vaccination registry records. However, subjects' recall of influenza vaccine history can be inaccurate, particularly for parental report of a child's vaccine history.⁷¹⁻⁷⁵ Eliciting self-reported vaccine history from subjects involves an interaction between subjects and research staff. A measurement error can arise due to researchers not being blinded to subjects' case status (in a case-control study) or due to subjects' desire to please the researcher. An additional limitation of self-reported vaccination is that subjects' recall will not capture information on the vaccine product received, if vaccines from multiple suppliers or of multiple formulations are available in a geographic region (although this would only be a concern where finer exploration of vaccine product-specific VE estimates are desired). Due to these limitations, WHO recommends against the use of self-reported vaccination history as the sole source of vaccination data.

Any method of assessing influenza vaccination history in an observational study has potential pitfalls and limitations. Ideally, influenza vaccination programmes, particularly new ones, should ensure a systematic way to record vaccine history so that the information is easily accessible. If record-keeping related to influenza vaccinations can be designed with subsequent observational studies in mind, the likelihood of successfully executing those observational studies will be much greater. In any event, it is essential to conduct pilot testing methods for collecting vaccine history before beginning any influenza VE study. This pilot testing should assess the accuracy and completeness of any proposed method for measuring vaccination history, and study protocols should be adapted accordingly. In some cases, incorporating multiple approaches for assessing vaccination status may be the best way to ensure that vaccine

information is complete and accurate. The manner of exposure ascertainment and the results of pilot testing should be reported to allow readers to put the VE results into context and assess the strength of potential biases. In particular, these results should include the sensitivity, specificity, and positive/negative predictive values of the proposed approaches to defining subjects as vaccinated or unvaccinated.

Recommended background research on vaccination history to inform VE studies:

- 1) If a proposed study is to rely on a vaccination registry, pilot testing should assess the ability of study staff to link study subjects to the registry, and should attempt to evaluate the accuracy and completeness of the registry data.
- 2) If a proposed study is to rely on vaccination cards, pilot testing should assess the ability to review the cards and the legibility and validity of the data on the cards.
- 3) If a proposed study is to rely on self-reported vaccination history, pilot testing should validate self-reported vaccinations relative to some external standard. All studies should assess vaccine coverage among participants (notably controls) in relation to expected values in the source population as a check on possible bias in vaccination status.

5. Measuring covariates

In addition to measuring outcomes and ascertaining vaccination history on all VE study participants, investigators will also need to collect data on other covariates. One purpose of measuring covariates is to stratify VE estimates based on sub-populations of interest. Depending on the objectives of the evaluation, existing influenza VE programmes often produce stratified VE estimates, e.g. by age group.^{20,68} Other groups of interest might be pregnant women or persons with certain comorbidities.

The other main purpose for collecting covariate data is to measure and control for potential confounders, either in study design (e.g. by matching) or in data analysis. In observational VE studies, confounding due to differences in disease risk or in care-seeking behaviour between vaccinated and unvaccinated subjects can greatly bias VE estimates. In an extreme case, studies using all-cause mortality as an outcome and without measuring true confounding factors may produce VE estimates that are ten-fold greater than what is expected or even plausible.^{58,59,76-78}

The important confounding factors to measure will depend on the outcome being studied, the study setting, and the target groups of the vaccination programme being studied. Specific variables to measure will represent a compromise between what is theoretically important to measure and what is possible to measure given constraints of time and resources. In influenza VE studies, strong confounders have often been factors that are not readily measured using pre-existing data sources such as administrative health-care databases.⁵⁸ This is particularly true of so-called “healthy vaccinee” effects in older adults, in which frailty is associated with higher risk of outcomes and lower probability of vaccination, while being difficult to measure

Bias: Systematic deviation of study findings from true associations in the source population.

Selection bias: Bias resulting from differences in enrolling or in collecting data on study subjects based on vaccination history or outcome status.

Confounding: Bias arising when exposure and outcome share a common cause that is not taken into account in analyses.

Covariates: Factors other than outcome and vaccination history that are measured on all study subjects. Examples include age, sex, and comorbid illnesses. Covariates are used to produce stratified VE estimates and to control for confounding. Detailed reporting of participant profiles based on key covariates also supports understanding of the applicability of results across seasons/settings and by comparison to expected values in the source population supports assessment of possible selection bias.

Potential confounder: A covariate associated with both vaccination and outcome and not caused by either. Confounding in VE estimates may be reduced by including potential confounders in statistical models of VE.

using health-care databases.^{61,76} Because of this, measuring important confounders will likely be time-intensive, requiring either interview of patients or review of medical records. WHO recommends pilot studies to identify factors that are strongly associated with vaccination and with disease risk prior to beginning any influenza VE study in a new setting. This formative research will be essential to the successful conduct of influenza VE studies.

To guide the selection of covariates to be measured, a number of potential confounders that have been considered in high-resource settings are described below. Factors that may be important predictors of both vaccine receipt and of care-seeking among persons with ARI are also included. The specific covariates to include will depend on the locale and context of a particular study. For illustrative purposes, the following list separates the key covariates, which have almost universally been found to be important for VE studies, and other covariates, which may be useful for specific settings.

5.1 Key covariates

Age is almost certainly a confounding factor, as both vaccine coverage and risk of influenza virus infection vary by age. Age is also an important stratification factor for VE estimates, as VE may differ in different age groups. Stratification of VE estimates allows researchers to assess the presence of confounding by age or of true difference in VE by age (i.e. effect modification).

Sex has not often been a confounder in studies conducted in high-resource settings,⁸ but may be more strongly related to health care utilization and vaccination in other settings.

Race/ethnicity is correlated with health care utilization in many parts of the world.

Date of symptom onset is an important covariate for characterizing the influenza epidemic in the population. It is needed in cohort studies to calculate person-time at risk, and in case-control studies to sample controls (if using incidence-density sampling).

Calendar time is a key covariate in test-negative studies (described in Section 5), because non-cases which are enrolled outside of an influenza season must be excluded from analyses to avoid bias.^{79,80} Calendar time is also correlated with vaccine uptake and with incidence of influenza, creating potential confounding by calendar time, although this confounding may not be meaningful in some settings.⁸¹ Date of symptom onset may be used to adjust for potential confounding by calendar time. It is important to note that in regions with temperate climates and well-defined influenza seasons, it is straightforward to determine which subjects to include and which to exclude. In regions with tropical climates where influenza may have multiple peaks, it can be more difficult to know when influenza virus is not circulating widely. At a minimum, calendar time should be considered as a confounder, and sensitivity analyses should compare VE estimates including all subjects vs only those enrolled during influenza peaks.

Time from symptom onset to specimen collection may be associated with the sensitivity or specificity of influenza testing. Stratifying VE estimates by time from onset to collection can help assess whether this may lead to biased estimates.

Use of antivirals is a confounder as patients who have used antiviral medicines either for treatment or for prophylaxis are more likely to have false negative test results. These patients should be excluded from study enrolment.

5.2 Other covariates

Receipt of other vaccines, such as pneumococcal vaccines, may be a marker for care-seeking behaviour and/or propensity to seek influenza vaccination. For studies using pneumonia as an outcome, receipt of pneumococcal or Hib vaccines should be measured as potential confounders, as the risk of pneumonia is lower in those who have received these vaccines, and their use may be correlated with influenza vaccination.

Prior history of influenza vaccination, although potentially difficult to measure accurately in many settings, is useful to measure, as receipt of prior years' influenza vaccine may impact the effectiveness of the current season's vaccine.

Presence and severity of cardiac or pulmonary comorbidities are likely important confounders. Persons with chronic cardiac or pulmonary disease are at increased risk of influenza-associated complications if they are infected, and therefore more likely to become cases in a hospital-based study. In high-resource settings underlying disease is also correlated with receipt of influenza vaccine, although in a non-linear fashion.^{63,77} Pilot studies on the association of underlying disease and receipt of vaccine will be essential for identifying particular aspects of underlying disease to be measured.

Functional and cognitive limitations have also been shown to be important confounders in VE studies among elderly adults in high-resource settings and particularly in relation to serious outcomes (i.e. hospitalization).^{58,77} Their role in other settings and age groups is less clear, and pilot studies would be useful.

Immunocompromising conditions have generally been uncommon among subjects included in VE studies in high-resource settings and so have not been important confounders. However, in settings where the prevalence of HIV/AIDS is high, HIV/AIDS may be an important confounder to measure.

Socioeconomic status is likely to be highly correlated with vaccination and with health-care-seeking behaviour, and should probably be measured. This will be an important area for pilot studies. Some proxy measures, such as household crowding or number of children in the household, may prove useful, as crowding may capture both some degree of socioeconomic status and risk of influenza virus infection.

Distance to study hospital/clinic may be correlated both with access to vaccination and access to medical care.

Access to medical care will be population-dependent. In some settings, availability and use of health insurance may affect patients' ability to seek care at certain facilities.

Other covariates which may be useful include:

Measures of outcome severity, such as duration, subsequent hospitalization (particularly for outpatient outcomes), or death, may be useful for assessing whether influenza vaccine reduces severity of outcomes in the vaccinated (although this is complicated to estimate).⁸² However, because these occur after the onset of the outcome, they are not potential confounders and should not be included as covariates in VE models.

Recommended background research on covariates to inform VE studies:

- 1) Pilot studies should determine the key predictors of influenza vaccination in the proposed study population. Is vaccination influenced by geography, socioeconomics, comorbidity, pregnancy, and/or age?
- 2) Pilot studies should determine the key predictors of care-seeking among persons with influenza or ILI. What factors influence decisions to seek care, and the facilities at which care is sought? Does care-seeking vary by geography, socioeconomics, comorbidity, pregnancy, and/or age?
- 3) Publishing the results of these pilot studies will increase the confidence of readers that any VE study in a new population has carefully assessed potential confounding factors before embarking on VE estimation.

6. Study designs

Observational VE studies use a variety of designs, including cohort designs, case-control designs, and pseudo-ecologic designs such as the screening method. This section reviews different designs for estimating influenza VE, and their strengths and limitations.

6.1 Cohort studies

A cohort study is perhaps the most familiar and intuitive observational study design. In a cohort VE study, subjects are first separated into vaccinated and unvaccinated groups. In each of these groups (or cohorts), the researchers subsequently attempt to identify all persons who have the outcome of interest. The incidence rate ratios or risk ratios are then estimated by comparing the incidence of outcomes among the vaccinated to the incidence of outcomes in the unvaccinated groups. The risk/rate ratios are then used to estimate VE:

$$VE = 100\% * \left(1 - \frac{I_v}{I_u} \right)$$

where I_v is the incidence (either incidence rate or cumulative incidence) in the vaccinated group and I_u is the incidence in the unvaccinated group.

Cohort studies have several advantages relative to other observational study designs. Due to the intuitive nature of the design, results of cohort studies can be relatively easily communicated to policy-makers and other stakeholders. In addition, because cohort studies can directly estimate incidence rates, these studies can be used to estimate the burden of influenza in the vaccinated and unvaccinated populations and to estimate the number of cases averted by vaccination. Particularly in high-income countries, it is often possible to define cohorts and identify influenza vaccination history and certain covariates through electronic databases from national or corporate health plan membership.

Cohort studies also have some important limitations with regard to the estimation of influenza VE, as follows:

- i) Researchers need to be able to enumerate cohorts of vaccinated and unvaccinated subjects. For prospective cohorts (where cohort members are identified prior to vaccination), the research team must be able to identify which cohort members may subsequently receive vaccine. For retrospective cohorts (where cohort members are identified after vaccination), the research team needs cohorts of vaccinated and unvaccinated persons in which vaccine status has been determined independently of any influenza outcomes.
- ii) Researchers must be able to identify the study outcomes in both the vaccinated and unvaccinated cohorts. This can be particularly challenging if study cohorts are drawn from large urban populations, where cohort members could seek care from a number of different providers or institutions. Careful thought must be given to the selection of cohort sites to ensure that there is a high likelihood that cohort members with the study outcome (such as hospitalization for laboratory-confirmed influenza virus infection) will be identified. It is particularly problematic if the probability of an outcome being correctly identified differs between the vaccinated and unvaccinated cohorts. As an extreme example, if the vaccinated cohort members are all selected from vaccinated persons in a semi-rural region with only one or two hospitals, while unvaccinated persons are taken from an urban region with many hospitals, hospitalizations will be more easily identified for the vaccinated subjects; failure to identify hospitalizations in unvaccinated subjects will bias VE estimates downward.
- iii) As with all observational studies, cohort studies are susceptible to confounding due to differences in vaccinated persons compared to unvaccinated persons. Confounding due to differences in health-care seeking behaviour, or to differences in risk of severe disease, can be substantial and can completely overwhelm a true vaccine effect.⁷⁸
- iv) Another limitation for cohort studies is the expense involved. Most of the outcomes of interest (such as hospitalization for laboratory-confirmed influenza) are relatively rare. A cohort study will have to involve a large number of subjects, generally several thousand in each cohort, in order to detect a statistically significant effect of vaccination. When coupled with the expense of correctly identifying vaccination history on all study subjects and of conducting thorough follow-up for events, the cost of a well-designed cohort study can approach the cost of a randomized trial. Given that a randomized trial is much less susceptible to confounding, there is little reason to conduct cohort studies of influenza VE, unless they can be built onto existing population-based studies, or use a special population for which vaccination status and study outcomes can be identified accurately. For example, a study of monovalent influenza A(H1N1)pdm VE among health-care workers in Kenya⁸³ found that even with easily identifiable outcomes and exposures, cohort studies are still prone to biases due to differences in health-care seeking between vaccinated and unvaccinated subjects.

6.2 Case-control studies

Case-control studies are also commonly used to estimate influenza VE. In a case-control design, researchers first identify individuals who experienced the outcome of interest (cases), and a comparison group of individuals who did not experience the outcome of interest (controls). Vaccination history is then determined for all cases and controls, and the odds of vaccination in each group is calculated. VE is estimated from the ratio of these odds:

$$VE = 100\% * \left(1 - \frac{O_{cases}}{O_{controls}} \right)$$

where O_{cases} is the odds of vaccination among the cases, and $O_{controls}$ is the odds of vaccination among the controls.

The primary advantage of case-control studies is that they are more efficient than cohort studies in terms of the number of study subjects required. Health-care encounters for influenza (and particularly for severe complications of influenza) are rare enough that a cohort study may require dozens or even hundreds of subjects to identify each case. In contrast, a case-control study may only need three or four controls per case to achieve comparably precise VE estimates.

Case-control studies also present important challenges:

- i) Misclassification of vaccine history is an important source of bias in case-control studies. A prospective cohort study identifies population members based on available vaccination history data, reducing the risk of misclassifying vaccine history. Because subjects are not identified and enrolled on the basis of vaccination status, case-control studies require investigators to determine vaccination history retrospectively for all cases and controls after they have been enrolled in the study. In the absence of registries or electronic medical records, it can be difficult to assess vaccination history accurately.
- ii) Selection bias is also a potential problem. In high-resource settings, where patients' vaccination history may be readily available to health-care providers and testing criteria are not determined by a study protocol, physicians may be more likely to order testing for influenza if the patients are unvaccinated. Studies of influenza VE should not allow influenza testing to be based on vaccination status. Ideally, testing of clinical specimens for influenza should be based on standardized case definitions and not on clinical decision-making which can introduce bias. If clinician-based testing is used, absence of bias should be demonstrated.
- iii) The choice of an appropriate control group is the greatest challenge in case-control studies. The controls should be chosen so that the distribution of vaccination is the same among the controls and in the population that gave rise to the cases. It can be very difficult to identify a proper control group. For instance, for a case-control study in which cases are patients hospitalized for laboratory-confirmed influenza, what is an appropriate control group? One common approach is to randomly select asymptomatic (and presumably disease-free)

individuals from the community around the hospital. However, the hospital may have a wide catchment area, such that hospitalized patients may come from a variety of communities, some at a considerable distance from the hospital. Selecting controls from the community around the hospital will lead to biased VE estimates if vaccine distribution or outcome risks differ across communities. Matching controls to cases based on community of residence can reduce this bias, but can be logistically challenging. Alternatively, hospital-based controls could be used by selecting inpatients at the same hospital who are hospitalized for a disease other than influenza. Such persons are likely to come from the same distribution of communities as the persons hospitalized for influenza, making them a more representative control group. Care must be taken in selecting these controls, however, because receipt of influenza vaccine may be correlated with receipt of other vaccines. The control group should not be hospitalized for a vaccine-preventable illness; otherwise, the prevalence of influenza vaccination in the controls will not represent the source population for the cases.

6.3 The test-negative design: a special instance of case-control evaluation

A third option for selecting controls, and one that is increasingly used for annual influenza VE estimation,²² is to use patients who meet the specimen collection criteria from the study protocol, are tested for influenza, and are found to have negative test results. In this approach, the target population for enrolment consists of all persons who seek care for a defined set of symptoms, typically ARI; cases are those with positive tests for influenza, and non-cases are those with negative test results. VE is then calculated as:

$$VE = 100\% * \left(1 - \frac{O_{pos}}{O_{neg}} \right)$$

where O_{pos} is the odds of vaccination among those testing positive for influenza, and O_{neg} is the odds of vaccination among those testing negative.

The test-negative design is predicated on the core assumption that influenza vaccine only protects against influenza, and has no effect on other non-influenza causes of ILI,^{79,84} a core premise that has been validated through randomized controlled trial data sets.⁸⁵ It is powerful for several reasons. Firstly, all cases and controls have sought care at the same facilities. Hence cases and non-cases will generally have come from the same communities, reducing bias due to community-level variations in vaccine coverage. Secondly, cases and non-cases have all sought care for similar sets of symptoms. This reduces confounding due to differences in health-care seeking behaviour between cases and non-cases, which is a major challenge to influenza VE studies. Vaccine status is typically collected and recorded at the time of specimen collection, prior to knowing the influenza test result, reducing the likelihood of differential exposure misclassification. Even with sensitivity for influenza detection as low as 70%, in the context of near-perfect specificity such as provided by influenza laboratory-confirmed by PCR, outcome misclassification has been shown to have negligible impact on VE estimates derived by the test-negative design.⁶⁶

While the test-negative design has a number of important strengths, caution is needed in the use of test-negative studies for influenza VE against severe outcomes, such as laboratory-confirmed influenza SARI. Numerous published studies have shown that test-negative designs effectively reduce confounding in studies of outpatient illness in high-resource settings. Similar data for test-negative designs for hospitalized outcomes are much sparser. As an example of one potential source of bias in test-negative studies, it is well known that underlying cardiac and pulmonary disease is strongly correlated with the likelihood of receiving influenza vaccination in high-resource settings. These underlying diseases also increase the risk of hospitalization for respiratory symptoms. If the symptoms used to identify patients to be included in a hospital-based study are too broad, the pool of non-cases could be biased towards persons with acute exacerbations of chronic conditions, who may have a biased distribution of influenza vaccination relative to the cases.

6.4 The screening method

The screening method is a pseudo-ecologic design, which uses individual-level data on vaccination history and other covariates from cases, and ecologic data on vaccination coverage in the population from which the cases came.⁸⁶ The advantage of the screening method is that it does not require detailed data collection on non-cases, which saves cost relative to a case-control study. However, studies using the screening method are fully dependent on accurate and valid data on vaccine coverage in the source population. It is also important to note that the source population for cases may differ from the general population in which the study is conducted, because of differences in access to care and health-care seeking behaviour or other reasons. This requires that the proper source population for the cases can be correctly identified and that vaccine coverage is properly estimated in this population. In many low-resource settings, vaccine coverage is calculated based on vaccine doses distributed and on estimated population size.⁸⁷

Because estimates of population size can be inaccurate due to seasonal migration and other factors, vaccine coverage estimates are often inaccurate when applied to a subset of the general population. Furthermore, it can be difficult to adjust for potential confounders using this design, as doing so requires separate estimates of vaccine coverage in population sub-groups defined by levels of the confounding factors. Studies using the screening method should only be undertaken in settings where vaccine coverage can be measured with high accuracy, which is a challenge in many settings. WHO therefore recommends against the use of screening method designs for estimating influenza VE.

7. Statistical considerations

7.1 Sample size

As with any epidemiologic study, influenza VE studies must be large enough to rule out chance as an explanation for the study results. In general, influenza VE studies are not simply intended to determine that VE is greater than zero, but to estimate the actual VE with some degree of precision. The sample size needed to estimate VE will depend on a number of factors, including the proportion of the population that is vaccinated, the incidence of the study outcome and its specificity for influenza, the expected VE, and the desired precision of the VE estimates. The effects that some of these factors can have on study sample size are illustrated in Table 1 and Table 2.

Table 1: Number of cases needed in a case-control study to detect the specified VE against laboratory-confirmed outcomes, assuming 30% of the population is vaccinated, 3 controls per case, a type I error rate of 0.05

Precision (± percentage points)	VE = 70%	VE = 50%	VE = 30%
20	109	215	351
15	192	370	612
10	412	823	1367

Table 2: Number of cases needed in a case-control study to detect the specified VE against laboratory-confirmed outcomes within ±10 percentage points at the specified vaccination coverage, assuming 3 controls per case, a type I error rate of 0.05

VE	5% vaccinated	10% vaccinated	25% vaccinated	50% vaccinated
10%	9424	4945	2315	1670
30%	6881	3532	1574	1033
50%	4590	2328	967	565

Table 1 illustrates that increasing the precision of VE estimates requires increasing the sample size. Estimating a VE of 50% within ± 20 percentage points requires 215 laboratory confirmed cases, while estimating 50% VE within ± 10 percentage points requires 827 laboratory confirmed cases. Table 1 also illustrates that, in general, the closer VE is to zero, the greater the sample size required to demonstrate statistically significant protection. Estimating VE of 30% within ± 15 percentage points requires 612 cases, compared to 370 cases if VE were 50%.

Table 2 illustrates that low vaccination coverage in the population of interest increases the sample size needed to detect VE, or to estimate VE with a given precision. To detect a VE of 30% (± 10) when 50% of the population is vaccinated would require 1033 cases in a case-control study. If only 5% of the population is vaccinated, detecting the same VE with the same precision would require 6881 cases.

Also of note is that sample size requirements specified in Table 1 represent what is needed to estimate average overall VE across (for example) all age groups and for all virus types and subtypes. Estimating age group, vaccine product, or type/subtype specific VE will require correspondingly overall larger sample sizes. For example, estimating VE of 50% within ± 10 percentage points in each of four age groups would require 565 cases in each age group, or 2260 cases overall. Furthermore, adjusting for confounders (via stratification or regression models) will also increase the sample size required to estimate VE with a specified precision.

7.2 Characterizing the study participants

Several descriptive analyses are useful for characterizing the study participants. Graphs of case counts over time are informative for understanding the seasonality of influenza in the target population.⁸⁸ These plots can also help in assessing whether calendar time is a potential confounder, particularly if coupled with plots of vaccine uptake over time. Flowcharts of the enrolment process can be used to identify possible sources of bias such as high rates of refusal to participate in the study.

Bivariate descriptive statistics are used to assess the distribution of covariates among the study participants. These are particularly useful for comparing covariate distributions between vaccinated vs unvaccinated subjects and between influenza cases vs non-cases. These bivariate comparisons aid in determining variables that may be included as potential confounders in adjusted VE estimates, by identifying variables that are associated with both vaccination and influenza disease. The bivariate distributions are also useful for comparing the study participants with participants in other influenza VE studies, as differences in age or comorbidities may contribute to different VE across populations.

7.3 Analytic approaches to estimate crude rate/odds ratios

In cohort studies, unadjusted (or crude) rate ratios can be obtained from the ratio of incidence rates of the study outcome between vaccinated and unvaccinated subjects. Unadjusted incidence rate ratios can be calculated using 2x2 tables or Poisson regression models. In case-control and test-negative studies, unadjusted odds ratio estimates can be obtained from the ratio of the odds of exposure among cases vs controls/non-cases. Adjusted odds ratios can also be calculated using logistic regression models.

7.4 Assessing potential confounders

In planning the analyses, certain variables will be included in the final VE models *a priori*, without examining their actual role as confounders. These variables may be chosen based on subject-matter knowledge that suggests they will be strong confounders, or for comparability with estimates from other populations.^{22,89} Age, for example, is associated both with vaccination and with risk of influenza, and so should be included *a priori*.

Beyond the variables selected *a priori*, researchers should determine which measured covariates are acting as confounders in the data. For this, the most appropriate method for the final selection of potential confounders to include in statistical models is the “change-in-estimate” approach.^{90,91} In the change-in-estimate approach, the unadjusted VE is estimated using an appropriate statistical model. VE is then estimated adjusting for a single covariate. If the adjusted VE differs from the unadjusted VE by more than a pre-determined percent, the covariate is considered to be a confounder and will be included in final models. A common threshold is to include covariates whose adjustment changes the crude odds ratio by 10% or more, but the threshold is at the researchers’ discretion. For example, if the crude odds ratio estimate is 0.56, after adjusting for calendar time, the adjusted odds ratio is 0.47. The crude estimate is $100\% * (0.56 - 0.47)/0.56 = 16\%$ higher than the adjusted estimate, therefore calendar time would be considered a potential confounder.

7.5 Final analyses

After identifying potential confounders, final VE estimates can be calculated. Generally, VE is estimated using regression models to adjust for covariates. Final estimates should include variables that are found to be confounders in the study sample as well as variables chosen *a priori*. As with any analyses, appropriateness of the statistical methods should be reviewed with a statistical expert with respect to model diagnostics and validity checks (e.g. goodness of fit, identification of outliers, and assessment for multiple co-linearity).

7.6 Pooling data from multiple VE studies

As noted in the sample size calculations, large sample sizes are needed to estimate VE against specific influenza types/subtypes or to estimate VE separately for different vaccine products or age groups. One approach to enable stratified estimates by age, vaccine product, or by virus type/subtype is to pool data from a single study setting across multiple influenza seasons. This approach has been used by the US Flu VE Network, for example, to estimate the relative effectiveness of live attenuated vaccines vs inactivated vaccines in young children.²⁶

An alternate approach is to pool data from separate VE studies conducted in different countries within a region. This can be through meta-analysis of reported VE estimates, or through the pooling of individual level subject data. In either case, pooling data has several challenges that must be considered:

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- i) The studies being pooled need to be measuring the same vaccine effect. The protocols must be sufficiently similar in terms of case definitions, exclusionary criteria, sampling timeframes, and vaccinations under study to make pooling meaningful. For example, it would not be meaningful to pool data from a hospital-based VE study with data from an outpatient-based study, as VE against influenza hospitalizations is unlikely to be the same as VE against outpatient illness.
 - ii) The studies to be pooled must have similar data available on key covariates to include in adjusted VE models.
 - iii) Perhaps most importantly, the study settings must be similar enough that the pooled results can be generalized to each study setting. This means that the populations under study must have comparable access to vaccination and to health care for acute respiratory illness. Influenza viruses may differ antigenically between different study sites, particularly when they are in different hemispheres or climate zones.

For these reasons, WHO cautions against pooling data from populations that are heterogeneous with respect to: influenza vaccine products, programmes or policies; health systems or care-seeking behaviours; or influenza infection risk overall or by type/subtype. This would apply to pooling data from special populations, such as those in prisons or nursing homes, with general community-dwelling populations. If pooled analyses are to be attempted, standard practices should be followed with regard to analysis of heterogeneity between populations.

8. Building a test-negative study onto SARI surveillance

One attractive approach to estimating VE in low-resource settings where influenza vaccine has been widely distributed is to build a test-negative study onto an existing sentinel hospital surveillance system. In this approach, patients hospitalized for ARI would be identified at surveillance hospitals as part of the existing SARI surveillance programme.⁹² Respiratory specimens (nasopharyngeal or oropharyngeal swabs or nasopharyngeal washes) would be collected from all (or a random sample of) the SARI patients. These specimens would be tested for evidence of influenza virus, preferably using RT-PCR. Study staff or surveillance staff would interview patients, their guardians, or other family members to assess influenza vaccination history, the course of illness, and important potential confounders. Vaccination history could be validated through medical record review, vaccine registry data, or other documentation. VE would then be estimated as $(1 - OR)$, where OR is the ratio of the odds of vaccination among SARI patients testing positive for influenza to the odds of vaccination in patients testing negative, adjusted for confounding factors.

This approach has the following advantages:

- i) It has severe disease as an outcome, which is of interest to policy-makers.
- ii) All cases are laboratory-confirmed, which makes it easier to detect a vaccine effect on disease and reduces confounding relative to studies that do not use laboratory-confirmed outcomes.
- iii) When SARI surveillance has already been established, most of the infrastructure needed for this type of study is already in place, although efforts to verify vaccination history from records or registries would need to be added.
- iv) The test-negative design reduces the strength of confounding by differences in health-care seeking between vaccinated and unvaccinated persons and by community variation in vaccine coverage.

Although this approach to VE studies has several advantages, it is important to stress that the use of the test-negative design has been much less used in hospital-based studies than in outpatient-based studies. It is even less studied in hospital-based settings in low- or middle-income countries where the patient base may differ from

that in more developed settings. Consequently, important sources of bias may not yet be well understood. Below are some considerations for conducting test-negative studies within SARI surveillance systems. It is highly advisable that the full study be conducted by scientists of sufficient epidemiologic expertise to address the complex issues discussed in this document during design and implementation of the study, and that a full study be preceded by pilot testing of methods for data collection, validating vaccination history, case identification, and laboratory testing.

8.1 SARI case definition

While WHO has proposed a SARI case definition,³⁹ the SARI definition used in practice may differ.^{54,93} Several aspects of the SARI case definition are important to consider if SARI surveillance is to be used for a test-negative study. SARI patients need to be identified soon enough that influenza testing remains highly sensitive. If subjects are enrolled after they are no longer shedding influenza virus, laboratory testing will falsely classify them as influenza-negative. These patients will be classified as non-cases in the analysis, biasing VE estimates downwards. Most existing test-negative studies restrict eligibility to patients whose onset was within the past seven days.^{20,94} To reduce the risk of false negative tests, SARI surveillance systems that use the WHO-recommended 10-day post-onset window should consider excluding from VE studies SARI patients with onset prior to the past seven days.⁵²

The specificity of the case definition also needs to be considered. A broad list of inclusionary signs and symptoms may increase the probability that the case definition will capture persons hospitalized due to influenza. However, a broad case definition also increases the contribution of other conditions to the SARI patient population. Test-negative studies of hospitalized patients that use broad case definitions can produce substantial differences in the prevalence of chronic conditions between cases and non-cases.³² This can introduce confounding by underlying health status.⁵⁸ If a test-negative study is based on SARI surveillance that does not use the WHO SARI case definition, the study participants should be restricted to SARI patients who have evidence of an acute infection and not some underlying chronic cause.

8.2 Laboratory testing

If a SARI surveillance platform is to be used for a test-negative VE study, another important factor is who in the surveillance system decides to test specimens from patients for influenza virus. Is laboratory testing based on a physician's clinical decision-making, or is it based on the use of case definitions by surveillance staff? Selection bias (discussed in Section 5) may be introduced if individual clinicians determine which patients are tested, as vaccinated patients may be less likely to have specimens tested than unvaccinated patients. Even if physicians' decisions are not based explicitly on vaccine history, clinical testing decisions are based on many factors that could exert subtle biases in a test-negative study.

Whether or not clinical testing results in a population sample that is biased with respect to vaccination history, clinicians are likely to preferentially order testing of specimens from particular subgroups of the population (such as young children). Relying on clinical testing would skew the VE study participants towards these

groups, which may or may not be the groups for which VE estimates are of highest interest. To avoid biases and sub-optimal populations for testing, the decisions on testing for SARI surveillance should be made by surveillance personnel and based on pre-defined case definitions.

8.3 Timing of enrolment

The seasonality of influenza varies considerably around the world, ranging from well-defined seasonal epidemics to year-round circulation.¹¹ Recruitment of subjects from SARI surveillance into a test-negative study should only be done during periods when influenza viruses are circulating in the population under surveillance. If subjects are recruited outside of an influenza season, they will almost invariably test negative for influenza and be treated as non-cases. Including non-cases from time periods when influenza is not circulating can lead to biased VE estimates, if influenza vaccine coverage varies between influenza epidemics and other time periods.⁷⁹ For example, in high-resource temperate settings, influenza vaccine is usually distributed in the autumn, prior to the winter influenza epidemic. If subjects are enrolled into a test-negative study in the autumn, nearly all will be non-cases. Because vaccine is still being distributed, vaccine coverage is lower in the autumn than in the winter. Thus, inclusion of these non-cases will lead to underestimation of the vaccine coverage relative to coverage during influenza season. In settings where influenza seasons are less well-defined, or where influenza may circulate year-round, then adjusting for calendar time (perhaps with indicator variables for calendar month) will be needed to control for differences in vaccine coverage over time.⁷⁹ But when influenza has a defined seasonality, adjusting for calendar time will mean that non-cases enrolled outside the influenza season will have few if any cases in their time strata, and will thus contribute little to the analysis, wasting resources used to recruit these subjects.

8.4 Other considerations

As with any observational study design, VE estimates from a test-negative study can be biased if vaccinated and unvaccinated people differ in the rate at which they come in contact with individuals infected with influenza virus.⁷⁹ In a test-negative design, this bias could be manifested if there is a localized influenza outbreak among a particular subset of the population, such as prison inmates or health-care workers at a specific facility. Vaccine coverage among these influenza cases is likely to be different than vaccine coverage in the general population, from which the test-negative subjects are drawn, leading to biased VE estimates. When conducting a test-negative study, researchers should be aware of localized outbreaks and assess whether to include patients from these outbreaks in the test-negative study.

9. Reporting influenza VE results

As with all observational studies, reports of influenza VE studies should include sufficient details on the study participants, data collection, and analyses to enable readers to judge the validity of the study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement provides recommendations for a minimum set of reporting elements.⁹⁵ These include descriptions of setting, dates of enrolment and follow-up, case definitions and exposure measurement, sample sizes and patients included/excluded, and key characteristics of the study participants. The STROBE guidelines provide a starting point for VE reporting. However, influenza vaccines are updated every year, with the aim of matching the continually changing viral strains, the relative prevalence of which varies geographically within a single season. Reports of influenza VE studies need to include additional information to allow readers to assess whether the study results can be generalized beyond the specific season and setting under study. WHO encourages investigators to share their raw individual-level, anonymised data from VE studies.⁸⁰ This would greatly aid pooling of results.

9.1 Description of influenza vaccines

To evaluate VE estimates, it is necessary to know which influenza vaccines and specific populations are being studied. At a minimum, this requires listing the virus strains that are included in the vaccine; for example, the northern hemisphere 2015/16 trivalent vaccine contained A/California/7/2009(H1N1)pdm-like virus, A/Switzerland/9715293/2013(A(H3N2)-like virus, and B/Phuket/3073/2013-like virus.¹⁶ In settings where a limited number of vaccine products are in use, the specific products should also be described, listing the manufacturer and product. Even in settings with a variety of vaccine products in use, efforts should be made to estimate the coverage with different vaccine types (live-attenuated vs inactivated, trivalent vs quadrivalent, standard dose vs high dose) among the study participants. It is generally difficult to enrol sufficient subjects to permit estimates of vaccine product-specific VE. However, including data on the relative frequencies of different vaccine products aids the comparison of VE estimates with other studies.

9.2 Description of circulating influenza viruses

It is also necessary to know the characteristics of influenza viruses that circulated in the periods during which VE was estimated. At a minimum, this includes describing the proportion of study subjects infected with A (H1N1), A (H3N2), B Yamagata, and B Victoria viruses. Therefore, in study settings where molecular characterization cannot be done, efforts should be made to transport influenza-positive samples to reference laboratories that can perform this testing. Because VE can vary by type, subtype, and lineage, these data allow comparison of VE estimates with VE estimates in other countries during the same time period. Ideally, these data should be coupled with antigenic characterization data from at least a subset of the viruses, as differences in VE across countries or settings could be due to the circulating of antigenically distinct viruses.

9.3 Stratification of VE estimates

An overall estimate of influenza VE against all viruses in the total population under study provides data on how well the vaccine worked in that setting, for that year. Generalizing the VE estimate to other years or settings requires a more thorough understanding of the season and its composition per type/subtype. This is done through stratifying VE estimates by virus and by age group. Unless circulating influenza is highly dominated by a single subtype or lineage,⁹⁶ VE estimates should be stratified by virus type, subtype, and lineage, assuming the sample size is sufficient. In settings where vaccine is distributed to multiple age groups, WHO recommends that VE estimates be stratified by age, as VE can vary across age groups. Common age groups for stratified VE estimates include children <9 years of age (for whom two doses may be needed to generate immunity when vaccinated against influenza for the first time), adults 18-49 years of age (for whom the highest VE is expected), and adults 65 years of age and older (for whom VE may sometimes be lower relative to other age groups).

In some settings it is possible to further characterize contributing influenza viruses genomically and antigenically.^{21,97} This enables interpretation of VE in relation to vaccine match/mismatch. In some instances, it may be possible to calculate VE specifically against viruses that are clade-level or antigenically matched/mismatched to vaccine. This is particularly relevant as laboratory proxies of vaccine match/mismatch to circulating influenza strains may be poor surrogates for anticipating actual vaccine protection. Linking genetic, antigenic and epidemiologic results may be informative in the short-term for explaining suboptimal vaccine performance during some seasons, in the intermediate term for guiding annual reformulation of vaccine, and in the long term for improving the understanding of genetic and antigenic markers of virus change and determinants/predictors of vaccine performance. It is recognized, however, that not all centres may have the capacity to directly link detailed laboratory findings to epidemiologic measures of vaccine performance and this may not be a priority undertaking in low-resource settings.

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Annex 1.

Glossary of terms

Antigenic drift	Constantly accumulating mutations in the HA and NA proteins of influenza A and B viruses which allows them to escape immune recognition, resulting in repetitive ongoing epidemic influenza outbreaks
Antigenic shift	Major changes in the influenza type A HA antigen, often with changes also in the NA antigen, caused by reassortment between different influenza A subtypes, such as between animal and human subtypes; such resulting viruses can potentially cause regional outbreaks or a global pandemic
Bias	Systematic differences in enrolling or in collecting data on study subjects based on vaccination history or outcome status
Case-control study	An observational study design in which subjects with (cases) and without (controls) the outcome of interest are identified, and vaccination history is assessed retrospectively
Cohort study	An observational study design in which study subjects are identified based on vaccination status and followed over time to identify incident outcomes
Confounding	Underlying differences in the risk of study outcomes between vaccinated and unvaccinated persons that lead to spurious vaccine effectiveness estimates
Covariates	Factors other than outcome and vaccination history that are measured on all study subjects; examples include age, sex, and comorbid illnesses; used to produce stratified vaccine effectiveness estimates and to control for confounding
Influenza-like illness (ILI)	A clinical syndrome defined by the World Health Organization as an acute respiratory infection with measured fever of $\geq 38^{\circ}\text{C}$, cough, and onset within the last seven days
Measurement error	A type of bias, in which subjects' outcomes, vaccination history, or covariates are measured incorrectly
Non-specific outcome	A study outcome that is defined from clinical signs and symptoms without laboratory confirmation of influenza infection
Outcome	A specific, measurable disease or health event; all study subjects should be at risk for the outcome, and the occurrence or non-occurrence of the outcome should be known for all subjects
Outcome misclassification	Incorrectly determining the occurrence of the outcome for a study subject; this includes falsely considering a diseased person to be disease-free, and falsely considering a disease-free person to be diseased

Potential confounder	A covariate that may reduce confounding in VE estimates when included in a statistical model
Screening method	A pseudo-ecologic study design, in which individual-level vaccination data are available on all cases but only population-level data on vaccine coverage is available for the source population
Selection bias	Bias that results when the probability of becoming a study subject differs for vaccinated vs unvaccinated persons (in a case-control study) or by likelihood of being identified as a case (in a cohort study)
Severe acute respiratory infection (SARI)	A clinical syndrome defined by the World Health Organization as acute respiratory infection with history of fever or documented fever of ≥ 38 °C, cough, onset within the last ten days, and requiring hospitalization
Source population	The larger population from which all study subjects are selected
Study participants	All persons enrolled in a VE study; outcomes, vaccine history, and covariates should be measured on all study participants
Test-negative design	A special type of case-control study design, where all study subjects meet some testing criteria and are tested for influenza; cases are those who test positive and comparison subjects are those who test negative
Vaccine effectiveness (VE)	Reduced risk of disease among vaccinated persons attributed to vaccination in real-world conditions; estimated from observational studies
Vaccine efficacy	Reduced risk of disease among vaccinated persons resulting from vaccination under ideal circumstances; estimated from randomized trials
Vaccine impact	Reduction in incidence of disease in a population where some members are vaccinated; usually estimated from ecologic studies



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