D4.1 Framework for analysis of influenza vaccine effectiveness studies

DRIVE

Development of robust and innovative vaccine effectiveness

WP4 – Framework for analysis and study reports

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Publishable Summary

The aim of this document is to describe a standard set of analytical methods that can be applied to measure IVE in the European context of diverse vaccine manufacturing, distribution, and administration as well as diverse influenza diagnostic and therapeutic approaches. This document will serve as guidance to revise the current protocols within DRIVE.

The different study designs that can be used to assess influenza vaccine effectiveness are presented, followed by aspects related to exposure and outcome definitions and collection of data. Sources of bias and confounders are explained and how they can be addressed through the design or the analysis. Laboratory tests that are available for the detection of influenza infections are described. Methods for rapid assessment of IVE are presented. From a data analysis perspective, methods for analysis of individual studies and pooled analyses (one-stage vs. two-stage pooling) are described.

Each chapter concludes with a set of recommendations, where appropriate, a distinction between studies collecting primary data (test-negative design studies) and studies that make use of secondary data (such as register-based cohort studies) is made. These recommendations serve as guidance for the ideal study through existing methods

List of abbreviations

AD-MA	Aggregated data meta-analysis
ADVANCE	Accelerated development of vaccine benefit-risk collaboration in Europe
ARI	Acute respiratory infection
CI	Confidence intervals
DFA	Direct fluorescent antibody
DRIVE	Development of robust and innovative vaccine effectiveness
ECDC	European Centre for Disease Prevention and Control
EHR	Electronic healthcare records
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunoabsorbant assay
EMA	European Medicines Agency
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
EU	European Union
HAI	Haemagglutination inhibition assay
I-MOVE	Influenza - Monitoring Vaccine Effectiveness
IFA	Immunofluorescent antibody test
ILI	Influenza-like illness
IPD-MA	Individual participant data meta-analysis
IVE	Influenza vaccine effectiveness
MAH	Marketing Authorization Holders
NATs	Nucleic acid-based tests
OR	Odds ratio
PCR	Polymerase chain reaction
POC	Point-of-care
RIDT	Rapid diagnostic tests
RR	Relative risk
RSV	Respiratory Syncytial Virus
RT-PCR	Real-time polymerase chain reaction
SAP	Statistical Analysis Plan

SARI	Severe acute respiratory infection
SHR	Single radial hemolysis
SVC	Shell viral culture
TND	Test-negative design
US	United States
VE	Vaccine effectiveness
VN	Virus neutralization assay
VPD	Vaccine preventable disease
WHO	World Health Organization
WP	Work package

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1 Background and objective

The main objective of DRIVE is to enable the collaboration of different public and private stakeholders to perform annual VE studies for the various influenza vaccines on the European market. Work package (WP) 4 aims to create a framework to analyse, present, interpret and report influenza vaccine effectiveness (IVE) study results in such a way that all stakeholders can endorse its conclusions. This document constitutes the deliverable of task WP4.1.

1.1 Purpose of this document

The aim of this document is to describe a standard set of analytical methods that can be applied to measure IVE in the European context of diverse vaccine manufacturing, distribution, and administration as well as diverse influenza diagnostic and therapeutic approaches. This document will serve as guidance to revise the current protocols within DRIVE. Please refer to D7.3 for novel or innovative methods; these will not be discussed in this document.

This set of methods builds upon existing guidance documents, such as IVE guidance from I-MOVE [1] and the World Health Organization (WHO) [2], and general vaccine effectiveness (VE) guidance from ADVANCE and ENCePP.

The new European Medicine Agency (EMA) guidance requires marketing authorization holders (MAHs) to estimate product-specific IVE against laboratory-confirmed influenza on an annual basis [3, 4]. Challenges faced when performing IVE studies and pooling results from those in different countries include bias and confounding, differences in strain circulation between EU countries, and differences in coverage affecting potential herd immunity. Additional challenges to be overcome when estimating brand-specific IVE include timely influenza-vaccine brand identification at individual level, adequate sample size and lack of uniform administration of vaccine types across populations.

This framework will be annually updated, mainly based on the input and feedback from WP2 on study tools and WP7 on pilot studies.

1.2 Contents of this document

Each chapter describes one aspect of IVE studies. At the end of each chapters, recommendations are provided for the “optimal” scenario; where appropriate, we distinguish between studies collecting primary data, such as test-negative design studies, and studies that make use of secondary data, such as data from healthcare databases. These

recommendations serve as guidance for the ideal study through existing methods. Importantly, however, following these recommendations are *not* pre-requisites for data analysed within the context of DRIVE, nor do they consider novel or innovative methods.

Chapter 2 *Study designs* reviews the different study designs that can be used for IVE studies and the pros and cons of each.
Chapters 3 *Exposure* and 4 *Outcome* describe data required for exposure and outcome ascertainment and issues of misclassification.
Chapter 5 *Potential biases and confounders expected across different settings* reviews potential biases and confounders, how they can be dealt with in the study design and what information is required to control for them in the analyses.
Chapter 6 *Optimization of the value of microbiological and virological information* describes characteristics of different laboratory tests, including their advantages and limitations.
Chapter 7 *Methods for rapid assessment of IVE* deals with methods and considerations for carrying out real-time or rapid IVE assessment.
Chapter 8 *Data analysis for individual studies* describes data analysis methods and considerations by study design.
Chapter 9 *Data pooling* reviews the advantages and limitations of data pooling and describes one-stage and two-stage pooling.
Chapter 10 *Overall recommendations* summarizes and integrates the guidance provided in all the chapters.

1.3 References

1. I-MOVE, Marta Valenciano, Alain Moren, Bernadette Gergonne, Camelia Savulescu. Methods for measuring Influenza vaccine effectiveness during influenza seasons and pandemics in the European Union. 2008.
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2 Study designs

Observational IVE studies use a variety of designs, with the cohort design and the case-control design being the most commonly used designs. There are different variations to these two core designs, with different strengths and limitations.

2.1 Cohort studies

The cohort design is a commonly used and intuitive observational study design. In this design, VE is estimated by comparing attack rates in vaccinated and unvaccinated subjects in a cohort at risk for the infection of interest. The unadjusted VE is calculated as

$$\widehat{VE}_{Co} = 1 - \widehat{RR}_{Co} = 1 - \frac{\widehat{I_v}}{\widehat{I_u}},$$

with the risk/rate ratio $\widehat{RR}_{Co}$ being estimated from the vaccine preventable disease (VPD) incidence in the vaccinated group ($\widehat{I_v}$) and in the unvaccinated group ($\widehat{I_u}$). The unadjusted VE can be calculated using 2x2 tables. Adjusted VE is typically estimated using Mantel-Haenszel method or using Poisson regression and variants thereof to account for overdispersion (quasi-Poisson, negative-binomial, zero-inflated negative-binomial regression) and/or clustering (random effects Poisson regression).

Cohort studies are intuitive, which makes it easy to communicate their results. Based on cohort studies, incidence rates can also be estimated, making cohort studies useful for burden of disease studies. However, cohort studies have strict data requirements with data on exposure, outcomes and potential covariates needed for the entire source population. Typical data sources for cohort studies are linked (vaccination) registers and electronic healthcare records (EHR) on primary and secondary care. In these cohort studies, secondary data is used from pre-existing databases, and the study protocols cannot affect the way the data is primarily collected, but only which data should be used and how to analyse the available data. There may also be prospective cohort studies with primary data collection.

Pros

- Intuitive design, easier to communicate results
- Can be conducted with secondary data (if of sufficient quality)
-

- Good for rare exposures

Cons

- Large operational sample (not a con when using secondary data)
- Data on exposure, outcomes and potential covariates needed for the entire source population (not a con when using secondary data)
- Subject to healthcare seeking bias
- Secondary data from pre-existing databases may be incomplete (e.g. in the case of influenza, lack of systematic laboratory confirmation)

2.2 Case-control studies

These designs compare vaccine exposure in cases to vaccine exposure in separate but 'comparable' controls. The big advantage over cohort methods is the reduction in operational sample size, though choosing suitable controls can be tricky and may introduce a selection bias. In case-control designs, researchers identify subjects who experienced the outcome of interest (cases) and a comparison group of subjects who did not experience the outcome of interest (controls). Then, for both cases and controls, the vaccine exposure history is assessed and VE is estimated as the ratio of the odds of exposure in cases and controls or:

$$\widehat{VE}_{CC} = 1 - \widehat{OR}_{CC} = 1 - \frac{\hat{p}_{case}/(1-\hat{p}_{case})}{\hat{p}_{control}/(1-\hat{p}_{control})},$$

with $\hat{p}_{case}$ and $\hat{p}_{contol}$ being the observed proportions of vaccine exposure in the cases and controls and with $\widehat{OR}_{CC}$ being the estimated ratio of odds of exposure in cases versus controls, which is equivalent to the odds of disease in the vaccinated versus unvaccinated. The unadjusted VE can be calculated using 2x2 tables. Case-control studies can be unmatched or matched by matching cases to controls on factors such as age, sex, chronic conditions and calendar time. Unmatched case-control studies are typically analysed using the Mantel-Haenszel method or unconditional logistic regression. Matched case-control studies can be additionally analysed using conditional logistic regression. However, analysing a matched case-control study using unconditional regression with adjusting for the matching factors in the analysis is generally recommended [5]. The logistic regression models can be extended to account for overdispersion (quasi-binomial, beta-binomial regression) and/or clustering (random effects logistic regression).

Pros

- Reduction in operational sample size compared to cohort studies

- No follow-up required
- Good for rare events
- Efficient in resources and time

Cons

- Choice of adequate controls may be difficult, as the vaccine coverage in controls should be the same as in the population that gave rise to the cases
- Selection bias may occur in the absence of sampling protocols
-
- Retrospective identification of vaccination status, which may result in lack of exposure confirmation (lack of validation of vaccination status can lead to misclassification)
- Rare-disease assumption required

2.3 Variants of the case-control design

There are several variants to the 'classical' case-control design. These designs are mainly different with respect to the way controls are selected.

2.3.1 Test-negative method

The test-negative design is the most frequently used design to estimate IVE. The design controls for selection bias due to health-care seeking behaviour by restricting the source population to patients who seek medical care for a respiratory illness. Participants are selected among individuals who seek care for disease syndrome likely associated with influenza such as acute respiratory illness (ARI) or influenza-like illness (ILI), who are subsequently subjected to confirmatory testing [6, 7]. The cases are then chosen among the test positives and the controls among the test negatives. The method may be useful to minimize selection and ascertainment biases, due to differences in (parental) attitude when seeking medical care and of physician differences in making decisions regarding laboratory diagnosis.
Within Europe, the test-negative case-control design has been frequently used by the I-MOVE network and the ECDC [8, 9].

Pros

- Reduction in healthcare seeking bias

- Reduction in ascertainment bias if sampling protocols are used and also due to collecting information on vaccination status prior to knowing the laboratory influenza test results
- Reduction in outcome misclassification.
- Can be integrated in existing influenza surveillance system
- Reduction in operational sample size to select and include the controls
- Can produce estimates comparable with those of case control and cohort studies in the presence of a highly specific diagnostic test [10]

Con

- Less suitable for secondary data unless sampling protocols are in place in routine care (TND nested in a clinical cohort)
- Inappropriate control groups or screening methods could lead to biased results
- Less intuitive to interpret the resultsx

2.3.2 Nested case-control studies

Nested case-control studies are case-control studies undertaken within cohort studies. Each incident case is matched with a number of controls sampled from the risk set for that case. The risk set usually comprises individuals who have not experienced the outcome event at the time of occurrence of the case (incidence density sampling). Nested case-control studies potentially offer great reductions in costs and efforts of data collection and analysis compared with the full cohort approach, with relatively minor loss in statistical efficiency [11].

2.3.3 Case-cohort studies

A case-cohort design is similar to a nested case-control design with cumulative sampling. Whereas in the nested case-control design the controls are randomly sampled from the non-cases, that is, those who did not get infected during the surveillance period, in the case-cohort design, controls are randomly sampled from the whole cohort, regardless of their disease status (case-base sampling). This has the advantage that in the case-cohort design the ‘rare-disease assumption’ need not to be made. Controls may include both cases and non-cases. The case-cohort design was proposed by Prentice [12] as a way of reducing the burden of data collection on covariates. However, as a result of the possible overlap between cases and controls proper standard errors are more difficult to obtain compared to the nested case-control design and require the construction of a pseudo-likelihood.

Pros

- Rare-disease assumption not needed

Cons

- Standard errors more difficult to obtain compared to nested case-control design

2.3.4 Case-coverage method with external coverage cohort (screening method)

The case-coverage or screening method uses data on the exposure prevalence in cases and in the coverage cohort, from which the cases originate [13]. The unadjusted VE is obtained as

$$\widehat{VE}_{SCREEN} = 1 - \widehat{OR}_{SCREEN} = 1 - \frac{\widehat{p_d}/(1-\widehat{p_d})}{\hat{X}/(1-\hat{X})},$$

with the odds ratio $\widehat{OR}_{SCREEN}$ derived from the vaccine exposure prevalence among the cases ($\widehat{p_d}$) and the, often externally-derived, estimate of the vaccine coverage in the coverage cohort ($\hat{X}$).

Both estimates $\widehat{p_d}$ and $\hat{X}$ are often available from routine surveillance, making the case-coverage method an inexpensive and ready-to-use method that might be useful in providing early effectiveness estimates or to monitor changes in effectiveness over time. Control for confounding is possible using stratified analysis, provided that the confounders are similarly measured for the cases and the coverage cohort. The method does not allow for uncertainty in the expected odds of exposure in the coverage cohort. This is immaterial when the coverage cohort is large, the major issue being the possible bias if cases are drawn from a population with a different vaccination profile from that of the coverage cohort. The method has been used to monitor IVE among elderly in Germany [14].

Pros

- Inexpensive and ready-to-use

Cons

- If multiple vaccines are used, it is not possible to determine IVE per brand/type, but only overall (unless brand-specific coverage data is available)
- Bias may be introduced if cases are drawn from a population with a different vaccination profile from that of the coverage cohort

- Difficult to correct for confounding (as this would require knowledge of vaccine coverage in subgroups with the confounding factors of interest)

2.4 Recommendations

For studies using primary data to monitor IVE, we suggest to use the test-negative case-control design, with an appropriate choice of control group and implementation of sampling protocols [15], or alternatively the prospective cohort design.

For studies using secondary data we suggest to use the cohort design.

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3 Exposure

In general, the exposure of interest is vaccination against influenza in the season under study (index season). The level of detail of the exposure definition depends on the study design and objectives.

3.1 Influenza vaccines

The recommendation on the composition of the seasonal influenza vaccines, i.e. the selection of the strains, is reviewed twice a year by WHO to adapt to changes in the virus' epidemiology, one for the Northern Hemisphere and another formulation for the Southern Hemisphere [16]. Influenza vaccines can be characterized by the influenza number of virus strains included, the vaccine type, the presence or absence of an adjuvant, the administration route and the production process [17] (Table 3.1).

Table 3.1. Characteristics of seasonal influenza vaccines available in Europe in 2017/2018 [17]

Characteristic	**Options seasonal influenza vaccines available in Europe**
Content	Trivalent (2 influenza A strains and 1 influenza B strain) Quadrivalent (2 influenza A strains and 2 influenza B strains)
Type	Inactivated/whole virus Inactivated/split Inactivated/subunit Live attenuated
Adjuvant	Without adjuvant With adjuvant
Administration route	Intramuscular Subcutaneous Intradermal Intranasal (live attenuated vaccines only)
Production process	Egg-based Cell-based Recombinant

3.2 Influenza vaccination and its special features

An influenza vaccination is the event that indicates the administration of an influenza vaccine. The person receiving the vaccination is thereafter considered vaccinated. WHO recommends vaccine-naïve children younger than nine years of age to receive two doses during the season when they first receive an influenza vaccine [2]. The full schedule consists of a single dose for all others. However, the immune response may need up to two weeks to be fully developed, therefore typically a person is considered immunized only at day 14 post-vaccination (after the

last dose). The immune response does wane with time [2]. Thus, one might want to take into account the time that has passed since vaccination, when studying its effect.

Influenza vaccination is recommended on an annual basis especially to those at high risk [18]. As a consequence, people can be repeatedly vaccinated against influenza over several seasons.

3.3 Data to be collected

Resulting from 3.2, the vaccine brand(s) and vaccination date(s) of all influenza vaccinations given during the index season should be collected to fully capture a person's vaccination history. Knowledge of the vaccine brand allows the deduction of the administered vaccine's characteristics (see Table 3.1) and subsequently the estimation of brand-specific effects. In a small number of cases, when there is only one brand per type, vaccine brand and characteristics can be inferred from the type. The exposure data should include the vaccination date, because it is important to know the timing of the vaccination within the season and in relation to the outcome. It might be also advisable to collect similar information on vaccinations received in previous influenza seasons. However, depending on the available data sources and the study design, the data requirements can be adapted and reduced.

3.4 Data sources

3.4.1 Administrative sources

Computerized or paper-based vaccination registers (see also WP2 task 2) originating from routinely recorded medical records might be the gold standard among the administrative sources providing individual-level data including the administered product and the vaccination date for all people covered by the register. Nevertheless, aggregated data, e.g. crude numbers of vaccine doses delivered in the target population, might also suffice for vaccination coverage estimation in a reference group of studies that utilize the screening method. However, unless vaccination coverage is brand-specific, brand-specific IVE cannot be calculated using the screening method in settings where multiple brands are used.

In some countries, the structure of the market may help to retrieve the vaccination brand of all those who received influenza vaccination in a specific area, such as countries or regions where only one brand is procured through tenders (see also D3.1 and D3.3).

In addition to the health care providers' medical record-based registers, also pharmacies, insurance companies, and vaccine manufacturers may provide (aggregated) data on

administered, sold, or distributed influenza vaccine doses, when possible (D3.1 and D3.3) [2, 19].

The brand name can also be deduced from the batch number (sticker on the vaccine vial) if collected.

3.4.2 Self-reporting

The initial information on vaccination can also be provided by the vaccinee. Self-reporting alone leads most likely to a dichotomous vaccination status without any details concerning the administered product or the exact vaccination date. Such data might suffice in studies that do not focus on brand-specific effects and only require the chronological order of exposure and outcome to be known. Vaccination cards held by the vaccinee might improve the information available to be collected through surveys and self-reporting, as could contacting the pharmacy or physician that provided the vaccine [2, 19].

3.5 Common exposure definitions

Common exposure definitions frequently used by the ECDC [20, 21], the I-MOVE/I-MOVE+ network [22, 23], and elsewhere are summarized hereafter.

The above protocols agree that an individual is considered ‘vaccinated’ starting from >14 days after last vaccination [20-23]. The first 14 days from vaccination are either also considered ‘unvaccinated’ (leading to two distinct exposure levels) [20-22] or they are considered ‘partially vaccinated’ (leading to three distinct exposure levels; this definition has been used in the DRIVE protocols) [23]. In either way, the effect measure of interest only compares the ‘vaccinated’ to the ‘unvaccinated’; ‘partially vaccinated’ vs. ‘vaccinated’ will be considered in sensitivity analyses. Depending on the study design, an individual’s exposure status might vary over time in follow-up and as such may be considered as a time-dependent variable (cohort) studies [20] or is assessed only once for the time point of the outcome’s occurrence in case-control studies and its variants [21].

If a (sub)-population, e.g. young children, is eligible for two doses in the index season, it is recommended to always use three levels of exposure. In addition to the distinction in ‘unvaccinated’, (‘partially vaccinated’), and ‘vaccinated’, individuals are classified to be ‘partially vaccinated’ for the period between the first and the second dose [20, 21]. In case of brand-specific analyses, exposure definitions are more strict considering an individual only to be ‘unvaccinated’ until the administration of any influenza vaccine and only to be ‘vaccinated’ if solely the influenza vaccine of the brand of interest had been administered [22, 23].

Influenza vaccinations in previous seasons, which could be also considered an exposure, are discussed as being a confounder (and effect modifier) in Section 5.2.8. If the effect of previous influenza vaccinations is of major interest, the exposure and covariate definitions could be interchanged by making vaccination in the index season the covariate and previous influenza vaccinations the exposure.

3.6 Exposure misclassification

Whichever data source is available or has been chosen, misclassification can occur. The consequences of exposure misclassification in terms of information bias are discussed in Chapter 5.

3.6.1 Adequacy of data source

When using administrative sources that record the presence of a vaccination but not a potential absence, it must be ensured that they are complete and cover the entire population of interest (i.e. the population from which cases and non-cases are retrieved). Otherwise, the part of the population not or incompletely reflected in the data is by default assumed to be not vaccinated. In case of aggregated vaccination information (when applying the screening method), it must be further guaranteed that this information originates only from the population of interest to avoid misclassification towards a wrongly increased number of vaccinated.

3.6.2 Data entry errors

As with any records, vaccination registers based on routinely entered medical records may contain data entry errors and consequently misclassification. Inaccurate records concerning the vaccinated subject, the administered product, or the vaccination date can lead to wrongly classifying study subjects as either not vaccinated or also vaccinated with a certain vaccine during the study period.

Incomplete or inaccurate data entry may result in misclassification. As the vaccination status in computerized vaccination registers is assumed to be “not vaccinated” and a single typo can break the automated process of correctly identifying the vaccination event (vaccinated subject, administered product, vaccination date) and cause misclassification, it seems more likely to classify the vaccinated as not vaccinated (rather than equally classifying the vaccinated as non-vaccinated and vice versa)[24].

3.6.3 Recall bias

Last but not least, self-reporting carries the risk of misclassification. The accuracy or completeness of recalling his/her vaccination history can vary e.g. depending on the study subject's age, and time since vaccination, and might be further influenced by the outcome status, especially if the subject or researchers conducting the interviews are not blinded, or if the outcome is related to compensations or other benefits. In the context of brand information, it may also be not known at all by the vaccinee. Complementing or verifying self-reported information through vaccination cards or administrative sources reduces this risk.

3.7 Recommendations

The following data on exposure should be collected, both for studies collecting primary data and those using secondary data:

- Vaccine brand(s) of all influenza vaccinations given during the index season (i.e. the season for which IVE is being estimated)
- Vaccination date(s) of all influenza vaccinations given during the index season, or if not available, the sequential order and relative timings of exposure and outcome
- How the vaccination status was ascertained and whether it was confirmed e.g. through medical records
- For children: how many doses they should have received based on vaccination in previous seasons (i.e. one dose if they have received influenza vaccination in an earlier season, two doses if they have not previously received influenza vaccination) and how many they actually received.

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4 Outcome

In general, in influenza vaccine effectiveness studies, the outcome of interest is the disease caused by an infection with an influenza virus, which can only be accurately confirmed by a laboratory test. However, it is rarely feasible to monitor the influenza infection status of the entire population of interest through an influenza season. Therefore, in practice, syndromic definitions (e.g., ILI, SARI) are also considered as surrogate to identify the subpopulation to be tested or to measure the vaccine effectiveness against a clinical syndrome likely associated with influenza.

4.1 Common outcome definitions

The only specific outcome is laboratory confirmation of the influenza virus [2, 25]. According to the EU influenza case definition, a laboratory confirmation requires the ‘isolation of influenza virus from a clinical specimen’, the ‘detection of influenza virus nucleic acid in a clinical specimen’, the ‘identification of influenza virus antigen by DFA test in a clinical specimen’ or an ‘influenza specific antibody response’ [26]. The laboratory tests currently available used to determine whether or not influenza infection has occurred are described in detail in Chapter 6. Laboratory-confirmed influenza infections in different settings, reflecting increasing levels of severity, may be considered, such as influenza in primary care, influenza requiring hospitalization, and admission to ICU following an influenza infection. VE frequently varies between influenza viruses; therefore, VEs are often reported by influenza A subtype or by influenza B lineage [27-29].

In contrast, influenza-like illness (ILI) and severe acute respiratory infection (SARI) are frequently used syndromic outcome definitions [2, 25, 30]. Normally ILI is used in primary care settings and SARI in hospital settings. WHO and EU case definitions of ILI and SARI are presented in Table 4.1.

Table 4.1. Case definitions of ILI and SARI by WHO and EU

Outcome	Institution	Case definition / clinical criteria
ILI	WHO	• 'an acute respiratory illness with a measured temperature of ≥38°C and cough, with onset within the past 10 days' [31]
	EU	• a 'sudden onset of symptoms' and • 'at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, or myalgia' and • 'at least one of the following three respiratory symptoms: cough, sore throat, or shortness of breath' [26]
SARI	WHO	• 'an acute respiratory illness with a history of fever or measured fever of ≥38°C and cough, with onset within the past 10 days, requiring hospitalization' [31].
	EU	• a 'sudden onset of symptoms' and • 'at least one of the following four respiratory symptoms: cough, sore throat, shortness of breath, or coryza' and • 'a clinician's judgement that the illness is due to an infection' [26].

The influenza disease outcome has also been defined for surveillance purposes based on diagnostic codes such as ICD-9, ICD-10, ICPC-2, or other codes such as Read codes in the UK, which are utilized in many patient information systems to classify a patient's diseases, disorders, injuries, and other health related conditions. Accordingly, a hospitalisation or a medical encounter would be considered to be due to influenza e.g. in presence of the ICD-10 codes J09, J10, J11, and all their subclasses [32].

Another outcome definition or common endpoint in observational studies has been death or all-cause mortality [2]. The suitability of this and other unspecific definitions like pneumonia is, however, questioned. Nevertheless, the use of such definitions should be further investigated, because even low IVE can have a large public health impact if the outcome of interest is serious or very frequent. Studies of IVE against unspecific outcomes must meet several demanding quality criteria including a large sample size to achieve a precise estimate that can be clearly distinguished from the null and elaborate adjustment for confounders which has been demonstrated to be an issue in several studies [2].

For case-control and TND studies, EMA guidance states laboratory-confirmed influenza should be the primary endpoint; based on the study setting (general population or hospital) secondary outcomes of interest may include pneumonia, influenza-related hospitalizations (influenza related or associated with respiratory or cardiac disease) or death [3]. For cohort studies, EMA

guidance states endpoints of interests may include medically attended respiratory infection (MAARI); medically attended ILI; all cause deaths; respiratory deaths; hospitalisations for pneumonia and influenza; hospitalisations for all respiratory conditions; laboratory-confirmed cases of MAARI/ hospitalised pneumonia and influenza and ICU admissions [3].

4.2 Data to be collected and data sources

4.2.1 Challenges

In practice, it is challenging to find a specific outcome definition that covers the whole disease burden due to influenza. The severity of the symptoms can vary a lot ranging from mild illness treated solely at home to very severe illness treated in hospitals' intensive care units and possibly leading to death. Further, influenza can make subjects more susceptible to secondary bacterial pneumonia and acute myocardial infarction or exacerbate chronic diseases. Then again, patients infected with other respiratory pathogens also circulating during the influenza season might present similar clinical syndromes [2].

4.2.2 Primary data to be collected

Consequently, aligning a non-specific clinical outcome definition like ILI or SARI with a laboratory test result is strongly recommended. The following data should be collected to optimally describe the endpoint of influenza test-positive, medically-attended ILI or SARI across all study designs:

- the symptoms forming the clinical syndrome of ILI or SARI including the information whether hospitalisation or intensive care treatment was required,
- the date of symptom onset,
- the date the respiratory specimen was taken,
- and the detected influenza type and subtype (if possible).

Such data could either arise from an active collection as part of a study designed to estimate IVE (e.g. TND or prospective cohort study), data sources for this could include medical records kept in hospitals or by GPs or from surveillance/ sentinel systems.

4.2.3 Secondary data collection

Nevertheless, this recommendation does not intend to generally exclude all other data collections from IVE estimations, especially from cohort studies through which less-specific

endpoints may be explored, although they might not provide as sensitive or specific information as indicated above. On the one hand, statutory infectious diseases registers covering clinically- and laboratory-confirmed influenza cases might provide outcome data that is less sensitive than that obtained through primary data collection (as not all potential cases will be routinely tested) but still highly specific. On the other hand, also for example diagnostic codes or data on antiviral drug prescriptions can be considered for deriving non-specific proxies for the outcome [19, 32]. Accordingly, information on the diagnostic code or antiviral drug describing the disease entity and the date of diagnosis or prescription approximating the disease onset should be collected. However, the resulting IVE estimates must be interpreted carefully (see 4.3 and 5.2.17). Additionally, outcomes that are not a proxy for laboratory-confirmed influenza (e.g. all-cause pneumonia) may be considered.

4.2.4 Incident and prevalent cases

Another thought to be considered is the possibility of repeated disease episodes. A second influenza infection in the same subject in the same season is rare but might happen. When the outcome definition is quite non-specific, the more frequently subjects might qualify again as cases in the same study. Especially when diagnostic codes or prescriptions are used to determine the outcome, an additional definition for a disease episode is required to distinguish frequently repeated events that are likely to belong to the same infection and sparse events that represent separate infections [6, 7]. The data collection should comprise all incident cases. While a physician involved in the collection of primary data might be able to differentiate between an incident and prevalent case, the timely distinction between these two is difficult when using secondary data as it strongly depends on the underlying coding and recording practices.

4.3 Outcome misclassification

Whichever outcome definition and data source has been chosen, misclassification can occur. The consequences of outcome misclassification in terms of information bias are discussed in Chapter 5.

4.3.1 Imperfect laboratory result

Imperfect laboratory test

Most of the laboratory tests currently available for detecting influenza infections are highly sensitive and highly specific but not perfect (see 6.1 and 6.2). Imperfect sensitivity bears the

risk of misclassifying diseased people as non-cases, while an imperfect specificity bears the risk of wrongly classifying non-cases to be influenza-positive.

Lag time between symptom onset and specimen

Typically, influenza virus shedding starts already a day before symptom onset and decreases substantially 4 days after symptom onset [33]. If a respiratory sample is taken thereafter, the virus may no longer be detectable, which introduces misclassification. Shedding peaks on the first 1-3 days of clinical illness [34-36]. Two studies on naturally acquired infection found the viral load of influenza B to be high for a longer time than for influenza A [35, 36].

Younger age has been associated with increased viral shedding (<16 vs. ≥16 years [37]; 0-5 vs. 6-15 vs. 16-64 years [38]), although this effect has not been consistently demonstrated across studies [36, 39]. No effect of vaccination on viral shedding among influenza cases was found [36, 37]. A systematic review on influenza A A(H1N1)pdm09 virus shedding found the duration of shedding to be increased with increasing disease severity and decreased by timely antiviral treatment [39].

Swabs

Preferred swabbing sampling for influenza detection are nasopharyngeal aspirates and washed, followed by nasopharyngeal swabs and mid-turbinate swabs; less preferred are throat swabs [40].

Use of antivirals

Antivirals like the neuraminidase inhibitors: oseltamivir and zanamivir and the M2 inhibitors: amantadine and rimantadine can be used for the prevention of influenza (e.g. as prophylaxis for individual exposed to influenza virus when admitted to hospital) and for the treatment of influenza in order to mitigate the associated complications [41].

The use of antivirals can reduce the duration of viral shedding [39], consequently respiratory samples taken after the start of antiviral treatment may already be negative, leading to misclassification.

4.3.2 Healthcare seeking behaviour

Influenza diagnosis is only confirmed after a health care visit and influenza infection cannot be ascertained in persons who do not seek care, yet the latter are assumed to not have been infected in cohort and classic case-control studies [7]. For this reason, it is important to specify the outcome is *medically-attended* influenza infection.

4.3.3 Adequacy of data source

If routinely recorded data on laboratory-confirmed influenza cases, diagnostic codes, or prescriptions are collected, their use in cohort studies estimating IVE must be reviewed carefully. In analogy to exposure data originating from administrative sources, it must be ensured that the records cover the entire population of interest, because study subjects without an entry related to the outcome of interest are considered disease-free. However, if the chosen data source's sensitivity to detect the outcome is imperfect and influenced by a study subject's vaccination status (e.g. due to differences in health care seeking behaviour or differences in the physicians' testing and diagnostic preferences), differential outcome misclassification occurs. Consequently, the proportion of diseased people misclassified as non-cases might differ between the different exposure groups.

4.3.4 Data entry errors

Registers based on routinely entered medical records are prone to data entry errors and consequently non-differential misclassification. Differential misclassification linked to the risk status of the patient can also occur, as it cannot be excluded that the content of the information entered and the scrutiny to encode this information is the same for an otherwise healthy subjects compared to a patient with underlying medical conditions.

4.4 Recommendations

In studies collecting primary data, the recommended outcome is influenza test-positive, medically-attended ILI or SARI. We suggest the collection of the following data points:

- Symptoms forming the clinical syndrome of ILI or SARI including the information whether hospitalisation or intensive care treatment was required,
- Date of symptom onset,
- Date the respiratory specimen was taken,
- Detected influenza type and preferably also subtype/ lineage for laboratory confirmed influenza.

In studies utilising secondary data e.g. from existing health care databases, the recommended outcome is laboratory-confirmed influenza, overall or stratified by clinical condition. However, this recommendation does not exclude the use of syndromic or unspecific outcome definitions discussed in 4.1, either in association with a positive influenza test or alone. We suggest the collection of the following data points:

- Clinical condition (if applicable),
- Date the respiratory specimen was taken,
- Detected influenza type and preferably also subtype/ lineage for laboratory confirmed influenza.

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5 Potential biases and confounders expected across different settings

This chapter describes biases and confounders that are important to IVE studies. A systematic literature review on confounding and bias is being performed as part of WP2 (D2.2). This chapter will be updated when the results are available.
If possible based on the data accumulated in systematic literature review, we will look into the impact of specific confounders on IVE estimates and add the results to future versions of this document, e.g. by ranking the different factors by expectation of their impact.

5.1 Definitions

A bias is a systematic error that leads to an incorrect effect estimate of the exposure on the outcome. Examples are selection bias and confounding.
A confounder is a variable that influences both the exposure and the outcome. Confounding can be subdivided into positive confounding, which leads to bias away from the null hypothesis (higher VE estimate), and negative confounding, which leads to bias toward the null hypothesis (lower VE estimate).
An effect modifier is a variable that differentially (positively or negatively) modifies the observed effect of the exposure on the outcome. Different groups have different risk estimates when effect modification is present [42].
It is noted that certain factors may both act as a confounder and effect modifier (see 5.3.3).

5.2 Biases and confounders

5.2.1 Health status

Persons who receive influenza vaccine may differ in important ways from persons who do not receive influenza vaccine, such as the presence of chronic underlying conditions (e.g. chronic pulmonary disease, cardiovascular disease, metabolic disorders, renal disease, treatment-induced immunosuppression and disease-induced immunosuppression) and frailty.
Here we differentiate between confounding by indication and the healthy vaccine bias (which includes confounding by contra-indication).

5.2.1.1 Confounding by indication (overall)

Confounding by indication occurs when a symptom or sign of disease is judged as an indication for a given vaccine, and is therefore associated to both with the vaccine and a higher probability

of an outcome related to the disease for which the vaccine is indicated/ or a specific brand is given [43]. In the case of influenza vaccination, patients who have underlying chronic conditions are more likely to be vaccinated as they are (perceived to be) at higher risk for (severe) influenza disease and risk groups are part of the vaccine recommendations; this can lead to a lower VE estimate [44]. Underlying conditions of interest include chronic pulmonary disease, cardiovascular disease, metabolic disorders, renal disease, treatment-induced immunosuppression and disease-induced immunosuppression [45]. Other risk groups mentioned in country-specific influenza vaccine recommendations should also be considered.

5.2.1.2 Healthy vaccinee bias and frailty bias

The healthy vaccinee bias and the frailty bias have the opposite effect of confounding by indication, i.e. vaccinated individuals may be healthier than unvaccinated individuals, leading to a higher VE.

Healthy vaccinee bias

Risk groups are part of the vaccine recommendations and are more prone to receive vaccination as compared to healthy subjects (see Section 5.2.1.1). However, within these groups recommended for vaccination (e.g. those based on age), those with a healthier lifestyle may be more likely to accept influenza vaccination.

Frailty bias

Vaccine coverage has been found to be low in frail patients, i.e. those with a low functional status and a high predicted probability of death during the upcoming influenza season [46, 47]. Healthcare providers may be reluctant to vaccinate such patients and likewise patients may "'give up' on preventive measures" [46]. Consequently, there may be relatively fewer severely ill patients in the exposed [45, 47].

5.2.1.3 Confounding by indication (type- or brand-specific)

Brand-specific IVE studies from settings in multiple countries will be pooled. Vaccine type recommendations may differ between countries. For example, country 1 may use non-adjuvanted trivalent vaccine for all adults, whereas country 2 may prescribe adjuvanted trivalent vaccine to a specific risk group and non-adjuvanted to the rest of the population. Furthermore, a healthcare worker may decide to vaccine with a specific brand (e.g. an adjuvanted vaccine, an intradermal vaccine, a quadrivalent vaccine) because he/she thinks a particular patient required additional protection. This may result in potentially important

differences between the exposed groups that affect the pooled VE estimate. Knowledge of vaccine recommendations or prescription practices across settings is necessary (also refer to WP2.2 and WP3.1).

5.2.2 Selection bias

Selection bias occurs when subjects are differentially enrolled in the study or analysis, or data is differentially collected based on their exposure or outcome status.

5.2.2.1 Selection bias based on vaccination status

In IVE studies, this can happen when not all subjects with acute respiratory illness are equally likely to be tested for influenza, especially in settings where testing takes place at clinician's discretion. For example, vaccinated subjects may less frequently be tested for influenza (due to perceived lower chance of influenza infection), which would lead to a higher VE estimate.

5.2.2.2 Selection bias based on outcome

Alternatively, the exact presentation of acute respiratory illness may – in the absence of clear sampling protocols - influence the clinician's decision to order influenza testing, which can either lower or increase the VE estimate [2].

Furthermore, in prospective studies with active enrolment for which informed consent is required (i.e. those taking place outside the context of routine surveillance or medical practice), individuals who are too ill to give consent (e.g. too acutely ill or because of worsened chronic condition) may not be enrolled, thereby biasing the outcomes captured to less severe disease. In addition, subjects that do not fulfil the ILI/SARI definitions are excluded from TND studies, thus excluding potential cases, such as e.g. those (elderly) with clear systemic symptoms and even radiologically-confirmed pneumonia, but without any signs of respiratory symptoms according to ECDC ILI definition.

5.2.3 Healthcare seeking behaviour

Not all subjects are equally likely to seek care, and hence to be diagnosed. Healthcare seeking behaviour is usually associated with exposure and the outcome (e.g. vaccinated persons may be more likely to seek medical care when diseased), and it may therefore lead to confounding. Multiple factors may influence healthcare seeking behaviour, ranging from funding of the healthcare system, to personal factors and disease severity.

Disease severity may be affected by exposure status (e.g. vaccinated persons may have less

severe disease, reducing their likelihood to seek care) and acute respiratory infection (ARI) aetiology. If ARI severity differs by aetiology, this might differentially impact the propensity to seek health care between cases and controls in a test-negative study [6].
Although healthcare seeking bias will be present in each setting, the bias is likely stronger in a GP setting than a hospital setting as patients with severe disease are more uniformly likely to seek care.

5.2.4 Information bias

Information bias arises when incorrect information about a variable is collected [19]. The most important types of information bias are exposure misclassification and outcome misclassification, although information bias is also applicable to other variables, such as confounders and covariates.
Exposure or outcome misclassification can be differential or non-differential. Misclassification is non-differential when the bias is the same for all subjects, regardless of exposure or outcome status. Non-differential misclassification usually causes a bias towards the null, leading to a lower VE. Misclassification is differential when the bias differs between exposed and non-exposed subjects, or between subjects with the outcome and subjects without the outcome. Differential misclassification can lead to bias towards or away from the null. Misclassification bias in vaccine effectiveness studies is discussed in detail by De Smedt et al. [48].

5.2.4.1 Misclassification of exposure

As described in more detail in Section 3.3, exposure misclassification can occur due to inadequate data source, data entry errors and recall bias.
Bias in the exposure due to inadequate data source or data entry errors, could be non-differential or in rare cases differential. Thus, in most cases it's expected to lower the VE. Data entry errors are most likely to result in subjects erroneously classified as unvaccinated, leading to a lower VE.

Recall bias can be differential or non-differential, therefore also here bias can go either way.

5.2.4.2 Misclassification of outcome

As described in more detail in Section 4.3, outcome misclassification can occur due to imperfect laboratory tests, lag time between symptom onset and specimen collection, lack of healthcare seeking (in cohort studies), use of antivirals, inadequate data source and data entry

errors.

Outcome misclassification due to imperfect laboratory tests, lag time between symptom onset and specimen collection, use of antivirals and data entry errors is most likely to be non-differential and thus underestimating VE.

Bias in the outcome due to inadequate data source can be non-differential or differential. In cohort studies conducted in the absence of a protocol or other measures ensuring a balanced case detection rate among the exposed and unexposed, differential outcome misclassification bears the risk of either under- or overestimating VE.

5.2.5 Age

Age is associated both with exposure (some risk groups recommended for vaccination are defined by age [49]) and the outcome (the very young are more likely to be infected due to lack of immunity, and very young and elderly are more likely to suffer severe influenza infection or to develop complications [50]). It is recommended that age is treated as an effect modifier (see Section 5.3.1), however within each strata, age can be treated as a confounder.

5.2.6 Gender/sex

Men and women may have different health care seeking behaviours, resulting in differences in influenza vaccine uptake and likelihood of medical consultation for influenza disease.

For example, a study conducted in Spain found that, within risk group recommended influenza vaccine, vaccine coverage was lower among women than among men [51].

Alternatively, in countries where health care workers are offered near compulsory influenza vaccinations or where pregnancy is considered an indication, women may have higher coverage.

5.2.7 Pregnancy

In many countries, pregnant women are recommended for influenza vaccination. Furthermore, changes to the immune system, heart and lungs during pregnancy makes pregnant women more susceptible to severe disease [52].

5.2.8 Smoking behaviour (or parental smoking behaviour)

Smoking may be positively associated with vaccination (e.g. smokers may have more chronic disease and therefore belong to the risk groups particularly eligible for influenza vaccination) or negatively associated with vaccination (e.g. smokers may be more prone to ignore health-

related recommendations). Furthermore, smoking may be associated with the outcome (e.g. disease may be more severe).

For children, parental smoking status must be considered. Wilson et al. showed that children hospitalized for influenza had more severe disease if they had been exposed to second hand smoking [53].

5.2.9 Socioeconomic status or applicable proxy

Socioeconomic status is a relevant variable to indicate access to health services. It may be associated with exposure in countries where influenza vaccination is not free, and with medically-attended outcomes in countries where healthcare is not free, or where insurance-based systems run in parallel to public care, and access to influenza vaccination in countries where influenza vaccination is not free.

5.2.10 Prior exposure to influenza vaccination and influenza infection

An important confounder is the prior exposure history of a patient, which includes both prior infection and prior vaccination status.

5.2.10.1 Prior influenza vaccination

Studies have found both positive and negative interference of repeated influenza vaccination on IVE [54], and this may differ by season. The antigenic distance hypothesis proposes that negative interference may occur if the consecutive vaccines are antigenically similar, and antibodies produced in the past season may neutralize vaccine antigens of the subsequent year's vaccine before it can trigger a full immune response, especially if the new circulating strain is antigenically different [55]. Conversely, positive interference may occur when the antigenic distance between the first vaccine and the new circulating strain is small, and pre-existing antibodies, are boosted by the response to the vaccine, helping to clear the new virus [55]. IVE may be influenced by vaccination patterns over at least several seasons [56].

5.2.10.2 Prior influenza infection

Prior influenza infection can influence the choice to receive influenza vaccine in the current year and can lead to a degree of existing immunity against influenza. Exposure to the virus is believed to induce lifelong cellular and humoral immunity that not only protects against infection by the original infective strain but may also provide cross protection against antigenically similar strains. This can lower the VE estimate in the current year. For example, Saito et al.

found a profound protective effect of medically attended influenza A infection in the prior season [57].

In the scientific literature, the prior influenza infection status is rarely taken into account due to the difficulty to collect accurate information. Furthermore, it is not known for how many previous years this information should ideally be collected.

Little research has been done on the interplay between prior influenza infection and prior vaccination and its effect on IVE [57].

5.2.11 Pneumococcal vaccination

Persons who receive influenza vaccine may be more likely to receive pneumococcal vaccine, due to overlap in recommendations for the two vaccines and because they may be offered during the same healthcare visit. Additionally, up-to-date pneumococcal vaccination can affect the occurrence of secondary bacterial infectious complications of influenza and is therefore an important confounder for non-specific outcomes; it would lead to a higher VE estimate. It is important to distinguish between 23-valent pneumococcal polysaccharide vaccine (PPS23) and 13-valent pneumococcal conjugate vaccine (PCV13) due to their differences in effectiveness against pneumonia.

5.2.12 Statins

Recent studies suggest statins may impair the antibody response and thereby reduce vaccine-induced protection [58]. Studies have shown reduced immune response to influenza vaccine [59], reduced VE to medically attended acute respiratory infection [60], and reduced IVE to some (but not all) influenza types/subtypes [58]. On the other hand, statins have been suggested to have a protective effect against infections [61].

5.2.13 Residence in long-term care facility

Residence in a long-term care facility may be associated with vaccination (e.g. residents of long-term care facilities are often vaccinated to minimize influenza outbreaks in the facility) and with the outcome (e.g. disease may be more severe due to underlying conditions, and influenza attack rate may be higher than in the community).
In relatively closed communities such as LTCF, disentangling the effect of individual protection and herd immunity caused by both care taking staff and visiting relatives being vaccinated may

be challenging [2].

5.2.12 Perinatal conditions

In addition to the variables for health status described above, a health status indicator for children based on birthweight and maturity at birth, as well as some other perinatal or congenital conditions (e.g. Apgar score or Down syndrome), is relevant for children. The cut-off age depends of the nature and severity of the condition.

5.2.14 Child's adherence to the local childhood vaccination programme

In case influenza vaccine is recommended for a child, either because the child falls within a risk group recommended for influenza vaccination or the country recommends influenza vaccine for all children, adherence to the local childhood vaccination program in general is likely positively associated with receipt of influenza vaccine and healthcare seeking behaviour.

5.2.15 Waning immunity

Intraseasonal waning of protection against influenza vaccination has been demonstrated through lower VE and geometric mean titres with increasing time since vaccination [62, 63]. Waning of immunity may occur at different rates for different vaccine components [62, 64], and may be of particular concern in adults aged 65 years and above [65].

5.2.16 Infection pressure

Influenza infection pressure varies between people. Factors associated with high infection pressure include being a healthcare worker, army conscript, childcare worker, and institutionalized individual (e.g. LTCF, prisons) and for children the number of siblings.
In certain cases, vaccinated and non-vaccinated subjects may have different contact patterns leading to a differential infection pressure [7]. For example, healthcare workers, who are frequently offered occupational influenza vaccine, may have increased exposure to influenza virus compared to the general population through contact with patients seeking care for influenza infection. In this example differential infection pressure would lead to a lower VE estimate.

5.2.17 Climatic factors

Influenza transmission is affected by climatic factors. Cold and dry conditions have been found

to favour influenza transmission [66-69].

Lowen et. al propose several mechanisms to explain the influence of humidity and temperature on influenza [69]. First, host may be more susceptible to respiratory virus infections as a result of desiccated nasal mucosa due to breathing dry air. Second, viral stability has been found to be very high at low relative humidity. Third, at low relative humidity, respiratory droplets carrying influenza virus are small (as water evaporated quickly), allowing them to remain airborne for a longer time, increasing the chance of transmission of the virus. Finally, viral shedding is increased at low temperatures. This could be due to reduced viral clearance as a result of slower mucociliary activity or increased viral stability.

5.2.18 Virus characteristics

Factors that pertain to the virus are virus virulence and level of antigenic match between the vaccine strain and the circulating strain.

Vaccine effectiveness is lower in seasons with a mismatch [70, 71]. Early news of a mismatch between the vaccine could potentially influence the level of vaccine uptake in a population. Furthermore, intraseasonal antigenic drift may cause variations in vaccine effectiveness within the season.

5.3 Effect modifiers

5.3.1 Age

Due to immunosenescence, which refers to the gradual decline of the immune system brought by natural age, vaccination in older adults is expected to lead to a lower immune response than in younger adults [72]. Furthermore, usually older adults are prone to develop more severe influenza disease or complications thereof. Both of these factors lead to lower VE estimates in older age groups [2].

Stratification of the data into age groups is advised. Harmonizing the age categories used in the analysis across studies will be crucial for the pooled analysis.

5.3.2 Immunosuppressed/ immunocompromised patients

Patients who are immunosuppressed or immunocompromised, whether as a result a disease or a treatment, are at risk for complicated influenza [73]. Immune response to influenza vaccine and although patients may still benefit from a degree of protection against severe disease, the VE in this population is lower than in patients who are not immunosuppressed/ immunocompromised.

5.3.3 Others

In addition to age, confounders listed above may sometimes be considered effect modifiers, such as prior vaccination/infection or health status (frailty). Frailty can be associated with vaccine effectiveness, especially in older adults and in risk groups. McGrath et al. state they could not control healthy-user bias through statistical adjustment, and therefore performed sample registration which reduced much of the bias [74].

5.4 Controlling for bias and confounding

The effect of bias and confounding can be controlled for at the study design level or in the analysis.

Table 5.1 shows for each bias or confounder described above what information would be required to control for it in the analysis and how it can be controlled for in the study design.

It will not always be feasible to address each type of bias or confounding. If data has not been collected, it is impossible to account for it in the analysis. For example, if the lag time between symptom onset and specimen collection has not been collected as part of routine clinical practice, this information will not be available from administrative databases and consequently it will be impossible to adjust for this type of outcome misclassification.

Some types of bias are best addressed through the study design, for example by including sampling protocols in TND studies to reduce selection bias. For many confounders, adjustment in the analysis (including matching in TND studies or the use of propensity scores in cohor studies) is likely sufficient. Separate analyses may be required across levels of effect modifier.

Table 5.1. Controlling for bias and confounding in IVE studies

Type of bias/confounder/effect modifier	Direction of bias (↓ or ↑ VE)	Controlling using study design	Information required to control in the analysis	Comments
Health status	↓: confounding by indication ↑: healthy vaccine bias, frailty bias	• Comparing groups with similar prognosis (e.g. between a GP who does and one who doesn't vaccinate patients- although this may be difficult in practice [44]; or across levels of immunosuppression or frailty status) • restricting or stratifying the study population (at the cost of reduced precision due to decreased sample size) [44], • individual matching of exposed and non-exposed into main prognostic strata or propensity-score matching (although this requires a large sample size) [44].	• Data on presence of chronic underlying conditions (like chronic pulmonary disease, cardiovascular disease, metabolic disorders, renal disease), treatment-induced immunosuppression and disease-induced immunosuppression, frailty • Number of hospitalization due to chronic conditions (as indicator for disease severity) • Data on presence of perinatal conditions (for studies in children only) • Measure of functional status/frailty (probably not captured in administrative databases)	
Selection bias	↓: less testing in vaccinated subjects ↓ or ↑: disease presentation	*Studies with primary data collection:* • Standardized case definitions and specimen collection criteria (same criteria used regardless of exposure status.) • Asking for consent from next of kin for patients too ill to give consent *Studies with secondary data collection:*		

Type of bias/confounder/effect modifier	Direction of bias (↓ or ↑ VE)	Controlling using study design	Information required to control in the analysis	Comments
		• Looking for selection in sampling according to vaccination/background factors (if also negative samples are available) • Using administrative databases avoids missing severely ill patients due to lack of consent		
Healthcare seeking bias	↓ or ↑	• Using TND minimizes this bias because both cases and controls have sought medical care for an acute respiratory infection [75].	• *Studies with secondary data collection:* Number of healthcare visits in a past set period of time • *TND:* Disease severity, in case this differs by ILI aetiology (e.g. requirement a medical visit, hospitalisation, ICU, leading to death or disabilities) [6]	• Bias more likely present in GP settings than hospital settings as healthcare seeking behaviour is likely more similar for more severe disease • VE then only holds for those people that would seek medical care for an acute respiratory infection • *TND:* Selection bias is still possible in TND IVE studies [10] However, Jackson et al. conducted a simulation study and concluded selection bias was only meaningful when rates of care seeking between influenza ARI and non-influenza ARI were very different; therefore selection bias is unlikely to be meaningful under conditions likely to be encountered in practice [76].
Misclassification (exposure)	↓: non-differential misclassification	*Studies with primary data collection:*		

Type of bias/confounder/effect modifier	Direction of bias (↓ or ↑ VE)	Controlling using study design	Information required to control in the analysis	Comments
	↓or ↑: differential misclassification	• Using the TND reduces differential recall bias as case status is unknown at time of recruitment [10]. • Studies using administrative database: • For sources that record the presence of a vaccination but not a potential absence, it must be ensured that they cover the entire population of interest. Otherwise, the part of the population not reflected in the data is by default assumed to be not vaccinated. • It must be assessed to what extent the vaccination records are expected to be complete. For example, GP records may not necessarily capture influenza vaccination at vaccination clinics or in occupational settings. • In case of aggregated vaccination information, it must be further guaranteed that this information originates only from the population of interest to avoid misclassification towards a wrongly increased number of vaccinated. • Checking interview data against vaccination registers or other data sources		

Type of bias/confounder/effect modifier	Direction of bias (↓ or ↑ VE)	Controlling using study design	Information required to control in the analysis	Comments
Misclassification (outcome)	↓: non-differential misclassification ↓or ↑: differential misclassification	• Choosing test with high specificity (more important than high sensitivity) because a non-specific testis expected to increase the proportion of false negatives [77] • Imperfect tests lead to less bias in cohort and case-control than in TND design, however trivial difference when using highly sensitive and specific test (e.g. RT-PCR) [77] • Considering inconclusive test results as negative causes less bias than considering these to be positive [77] *Studies with secondary data collection:* • it must be ensured that the records on disease status cover the entire population of interest, because study subjects without an entry related to the outcome of interest are not considered as cases	• Use of antivirals • Laboratory test used • Sensitivity and specificity of laboratory test. • Lag time between symptom onset and testing • For non-laboratory confirmed outcomes, an analysis in different periods of the season (peak or outer ends of season), where non-specific outcomes will likely be more specific in the peak period.	
Age		• For young children: to collect age in months	• Age as a continuous variable or in age groups [age groups to be harmonized!]	
Gender/sex	↓ or ↑		• Male/female from health care records	
Previous influenza vaccine (any) in past	↓ or ↑	• Yes/No from past health care records or interview	•	

Type of bias/confounder/effect modifier	**Direction of bias (**↓ or ↑ VE **)**	**Controlling using study design**	**Information required to control in the analysis**	**Comments**
seasons				
Previous pneumococcal vaccine in past years	↑: for non-specific outcomes (that include secondary bacterial infectious complications)		• Yes/No from past health care records or from interview	
Previous laboratory-confirmed influenza infection in past seasons	↓ or ↑		• Yes/No from past health care records	• Controlling for previous influenza infection is extremely difficult, as multiple factors are involved such as time since infection, influenza strain, diagnosis of past infection (depending on healthcare seeking behaviour and laboratory-confirmation), and availability of past health records.
Child's adherence to the local childhood vaccination program	↓ or ↑		• Receipt of childhood vaccines	
Infection pressure	↓ or ↑		• Healthcare worker yes/no • Army conscript yes/no • Childcare worker yes/no • Day care attendance (for pre-school children) • Number of siblings (for children)	• Controlling for differences in exposure to influenza virus is extremely difficult, as this variable is largely unobserved.
Pregnancy			•	•

Type of bias/confounder/effect modifier	**Direction of bias (**↓ or ↑ VE **)**	**Controlling using study design**	**Information required to control in the analysis**	**Comments**
Smoking / parental smoking status	↓ or ↑		• From health care records or interview (non-smoker, ex-smoker, smoker)	
Institutionalization	↓ or ↑		• Institutionalized vs. non-institutionalized • From health care records or interview	
Socioeconomic status (or proxy)	↓ or ↑			
Statins	↓ or ↑		• Use of statins	
Residence in long-term care facility	↓ or ↑			
Waning immunity	↓ over time		• Time since vaccination	
Virus characteristics	VE may change over time		• Calendar time (to account for any drift)	

5.5 Recommendations

For studies using the test-negative design, we suggest the collection of the following data points:

- Age (stratification by age groups, minimally 6 months-14 years; 15-64 years; 65+ years)
- Gender
- Chronic underlying conditions (e.g. liver disease, heart disease, diabetes, cancer, immunodeficiency/ organ transplant, autoimmune disease, lung disease, anemia, renal disease, dementia, history of stroke, rheumatologic diseases, obesity)
- Use of influenza antivirals (type, timing)
- Lag time between symptom onset and testing
-

Not all data points that can be collected through primary data collection are available in secondary data. For studies using secondary data, we suggest the collection of the following data points.

- Age (stratification by age groups, minimally 6 months-14 years; 15-64 years; 65+ years)
- Gender
- Chronic underlying conditions
- Past healthcare use (e.g. nr of GP visits, nr of hospitalizations)

Each study's SAP should explain how the data will be used in the analysis. Information on how to adjust for confounders in the analysis can be found in Chapter 8.

5.6 References

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6 Optimization of the value of microbiological and virological information

The WHO recommends the use of laboratory-confirmed outcomes as opposed to non-specific (syndromic) outcomes [2]. The decision to assess potential study subjects for laboratory-confirmed influenza virus infection should be based on pre-specified protocol guidelines, in order to avoid systematic misclassification of study subjects, which may arise in case clinicians are allowed to decide whom to test. Whenever possible, study protocols should specify the symptoms, duration of illness, and other eligibility criteria for attempting to enrol and test patients for influenza (in studies based on administrative databases this cannot be done). According to the Committee for Medicinal Products for Human Use Guideline on Influenza Vaccines, Non-clinical and Clinical Module [3], cases should meet the EU ILI and influenza case definitions. An influenza case definition that includes laboratory confirmation is essential to enable estimating IVE against influenza. Available laboratory tests to confirm influenza can be grouped in direct or indirect diagnostic tests.

6.1 Direct diagnostic tests

Direct diagnostic tests to identify influenza viruses are done on nasal or throat swabs, nasopharyngeal aspirates or bronchoalveolar washes [78]. Since the average duration of virus shedding in infected persons is around five days, and highest around the time of illness onset [79], the sample should ideally be collected within seven days after illness onset [2] (in studies with primary data collection), to reduce the likelihood of a false negative test result (otherwise sensitivity would be harmed). Sensitivity may be further improved by choosing non-cases from swabs testing positive for another respiratory virus, to ensure that the sample is of sufficient quality to detect virus [10] (alternatively, reference/housekeeping genes could be co-detected [80]).

6.1.1 Viral culture

Culture of influenza virus from a respiratory specimen represented the gold standard for diagnosis in the past. Results are available in three to 10 days; this reduces its utility for patient management. Shell viral culture (SVC) is another viral culture approach in use since the early 1990s. It consists of a rapid culture method that provides results in 24-48 hours. It involves propagation of viruses in mammalian cells grown in small 1-dram vials or shell vials, followed by staining with influenza virus-specific fluorescent monoclonal antibodies [81]. It has a higher sensitivity compared to the traditional viral culture technique. A modified SVC method using R-mix cells, a mixture of mink lung cells and human adenocarcinoma cells, has even higher sensitivity and a turnaround time of 1.4 days.

Viral culture is almost 100% specific and is nearly as sensitive as PCR when samples have high viral titers [82], e.g. in children, as children shed virus in higher titre and for longer periods of time than adults. Typically, the influenza virus-specific monoclonal antibodies used for virus detection in culture target the conserved NP protein, which allows distinction between influenza type A vs type B. However, this method cannot be used routinely since it is quite cumbersome, requires expertise, specialized equipment, and a large amount of time for testing.

6.1.2 Reverse Transcriptase-Polymerase Chain Reaction

The Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) has replaced viral culture as the gold standard test for laboratory confirmation of influenza virus infection during acute illness. It involves three essential steps:

i) Extraction of viral RNA from clinical specimens;
ii) Reverse transcription of viral RNA to a single-stranded cDNA using the enzyme reverse transcriptase; and
iii) Amplification of the PCR product coupled to fluorescent detection of labeled PCR products [81].

Beyond laboratory confirmation of the presence of influenza virus infection, RT-PCR provides a direct and complete identification of Influenza A viruses viral type and subtype and of influenza B viruses lineage, so it allows IVE estimate stratification by virus type/subtype or lineage. Nearly all RCTs use RT-PCR assays for influenza testing [83], based on the fact that it is currently the most sensitive (sensitivity is near to 100%, see Table 6.1 and Table 6.2; lower limit of detection, 1–10 infectious units [84]) and specific (specficity ranges between 91.1% and 100%) method for detection of influenza viruses [79, 85]. It has a 2% to 13% higher detection rate than viral culture and results can be obtained within hours [86]. Moreover, it enables appreciation of the genetic variability of influenza viruses.

The multiplex PCR identifies other viruses than influenza, the most important of which are respiratory syncytial virus A and B, rhinovirus/enteroviruses, and human metapneumovirus; followed by parainfluenza viruses, adenoviruses, coronaviruses and bocaviruses.

Potential disadvantages of RT-PCR are represented by the fact that it requires technical expertise and expensive equipment, and that its ability to detect the virus depends on the quantity of virus in a sample: the less virus, the more cycles required to identify influenza product. The US Centers for Disease Control and Prevention suggested using 37 cycles as the limit for classifying a sample as

positive, on account of the fact that positive results detected using a higher number of cycles may be false positives (reduced specificity) [79]. Finally, in case of novel virus appearance, rapid production and validation of new primer and probe sets may be required [84].

6.1.3 Rapid influenza diagnostic tests

A number of easy-to-use, rapid influenza diagnostic tests (RIDTs) have become available in recent years to detect influenza virus antigens or viral enzyme activity. Results are provided in approximately 15 minutes, and can be used at the point-of-care (POC) in a routine clinical setting (e.g. at the patient's bedside or at the physician's office).

A major downside is that RIDTs are unable to distinguish between influenza A subtypes or, for some tests, between influenza types A and B. Moreover they are less sensitive and also considerably less specific than RT-PCR, and can therefore cause disease misclassification [2]. According to the WHO recommendations on the use of rapid testing for influenza diagnosis, issued in 2005, the median sensitivity of rapid tests is 70–75%, lower than that of cell culture, while their specificity usually exceeds 90% (median 90–95%) [86]. A more recent metanalysis of 159 studies involving 26 RIDTs found that RIDTs have a high specificity (98.2%, 95% CI 97.5% to 98.7%) and positive likelihood ratio (34.5, 95% CI, 23.8 to 45.2) and modest and highly variable sensitivity (62.3%, 95% CI 57.9% to 66.6%) for detecting influenza [87]. Because of the low sensitivity, false negative results are a major concern with RIDTs, which therefore tend to underestimate IVE [88, 89]. The potential for false negative results is a concern especially during peak influenza activity. These findings mean that a positive RIDT result in a patient with ILI provides firm support for the diagnosis of influenza, whereas a negative RIDT result has a reasonable likelihood of being a false negative and therefore should be confirmed by other laboratory diagnostic tests (RT-PCR, viral culture, or immunofluorescence) [2].

According to Tanei et al., testing too early could increase false negatives, therefore RIDT should not be used soon after onset[90].

Since young children have higher viral loads and longer viral shedding than adults, RIDTs perform better in children, with approximately 13% higher sensitivity than adults. Similarly, RIDTs have a higher sensitivity for detecting influenza A, which causes more severe disease and therefore, usually, higher viral load than influenza B. In the metanalysis by Chartrand et al., no single commercial brand of RIDT performed markedly better or worse than the others; however, authors cautioned that head-to-head comparisons were not done in most studies [87].

Although specificity is high, false positive results can also occur, especially when influenza activity is low.

6.1.4 Detection of viral proteins

Detection of viral proteins by immunofluorescence, using direct fluorescent antibody (DFA) testing, also known as the immunofluorescent antibody test (IFA), or enzyme immunoassay (EIA), involves sedimentation of respiratory epithelial cells onto a slide well and subsequent staining with influenza-specific antibodies conjugated to fluorescent dye [84]. It is a rapid, relatively low-cost, and commercially available method. Results are available in 1-4 hours.

For seasonal influenza viruses, DFA sensitivity ranges from 60%–100%, compared with the traditional viral isolation procedures (Table 6.1. Synthesis of laboratory tests performances in terms of sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV).). The sensitivity of DFA tests for seasonal influenza appears to be higher than that of POC tests, however DFA requires technical expertise and the availability of a fluorescence microscope [84]. During the 2009 H1N1 pandemic, DFA tests sensitivity was variable, ranging between 38% and 93%, compared to RT-PCR-based approaches [34, 81, 91]. In the study by Bakerman et al. it missed one-third of infected individuals, as the reported test sensitivity was 65.0%; with addition of viral culture, sensitivity improved to 81.3% compared to PCR as the gold standard.

6.2 Indirect diagnostic tests

6.2.1 Serology on paired blood samples

The approach based on virologically-confirmed human influenza cases has the disadvantage that virus shedding in infected persons typically lasts only a week and has often diminished or ended by the time of sampling [92]. In addition, infections may cause only mild illness, leading to cases possibly remaining undetected. Studies based on the serological evidence of infection have a wider window of detection. Data, however, need to be interpreted with caution due to cross-reactivity of antibodies among and within virus subtypes and sensitivity decreases when used to detect antibodies against novel influenza subtypes [93].

Serology consists of collecting a first blood sample at symptoms onset and a second two to three weeks later. Influenza infection is defined by at least a 4-fold rise in specific antibody titer between paired sera. Antibody titer can be obtained by different methods. Those most commonly used are

the haemagglutination inhibition assay (HAI), microneutralization or virus neutralization assay (VN), single radial hemolysis (SRH), complement fixation assay, enzyme linked immunoabsorbant assay (ELISA) and Western blotting [81]. One major concern of ELISA-based tests is the lower sensitivity compared to nucleic acid-based tests (NATs). A novel europium nanoparticle-based immunoassay for rapid detection using monoclonal antibodies directed against the nucleoprotein from influenza A and influenza B viruses showed a sensitivity of 90.7% for influenza A viruses and 81.80 % for influenza B viruses with 100% specificity [94].

This test is unlikely to become relevant in DRIVE.

Table 6.1. Synthesis of laboratory tests performances in terms of sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV).

PUBLICATION	TEST	SE (%)	SP (%)	PPV (%)	NPV (%)	Notes
Baker man 2011 [95] Nasopharyngeal swabs	DFA	65.0 (59.0, 71.0)	99.6 (99.1, 100)	98.8 (97.0, 100)	85.9 (83.2, 88.7)	
Chartrand 2012 [86] viral culture and RT-PCR used as references	RIDT	62.3 (57.9-66.6) There was considerable overlap among the accuracy estimates for the RIDTs. Directigen Flu A had the highest pooled sensitivity (76.7% [CI, 63.8% to 86.0%]), followed by QuickVue Influenza test, although the difference from the overall estimate was not statistically significant. However, BinaxNOW, Directigen Flu A+B, and QuickVue Influenza A+B had a lower sensitivity compared with the overall estimate (57.0%, 57.2%, and 48.8%, respectively).	98.2 (97.5-98.7) Specificity was consistent among most RIDTs	34.5 (23.8-45.2)	0.38	POOLED ANALYSIS RIDTs had a significantly higher pooled sensitivity when compared with viral culture rather than RT-PCR because of the increased accuracy of the latter. Subgroup analyses showed that RIDTs had a significantly higher pooled sensitivity in children (66%, 95% CI 61.6% to 71.7%; 60 datasets) than in adults (53.9%, 95% CI 47.9% to 59.8%; 33 datasets); specificities were similar between the age groups. RIDTs were associated with significantly higher sensitivity for detecting influenza A (64.6%, 95% CI 59.0% to 70.1%; 72 datasets) than for detecting influenza B (52.2%, 95% CI 45% to 59.3%; 27 datasets). Results were unchanged when RIDT brand, specimen type and reference
Ganzenmueller 2010 [90]	Point-of-care	18.2	100	100	78.1	Detection of novel 2009 influenza A (H1N1)

Respiratory specimens (nasopharyngeal swabs, pharyngeal washes and bronchoalveolar lavage samples), using RT-PCR as gold standard	(POC) RIDT					
	DFA	38.7	100	100	82.2	
	Virus isolation	45.7	99.8	95.5	94.8	
Ginocchio 2009 [91] Detection of novel 2009 influenza A (H1N1)	DFA	47.2	99.6	90.6	96.2	Ages ranged from 4 days to 98 years; authors did not differentiate between adult and paediatric populations
	RIDT	21.2	99.5	76.5	94.5	
	Viral culture	98.4	100	100	99.9	
Hindiyeh 2005 [96] Throat and nasal swabs Viral culture as reference	Multiplex TaqMan	Influenza A: 100 Influenza B: 95.7	Influenza A: 91.1 Influenza B: 98.7	Influenza A: 84.7 Influenza B: 94.3	Influenza A: 100 Influenza B: 99	Authors concluded that the multiplex TaqMan assay is highly suitable for the rapid diagnosis of influenza virus infections both in well-established molecular biology laboratories and in reference clinical laboratories.
	Multiplex RT-PCR	Influenza A: 100 Influenza B: 100	Influenza A: 93.1 Influenza B: 98.3	87.8 Influenza B: 93.2	Influenza A: 100 Influenza B: 100	
	IF	Influenza A: 84.4	Influenza A:	Influenza A:	Influenza A:	

		Influenza B: 33.3	98.8 Influenza B: 100	97.2 Influenza B: 100	92.8 Influenza B: 86.7	
Kenmoe. 2014 [97] Nasal swabs RT-PCR as gold standard	RIDT	29.4	100	100	89.5	
Landry 2014 [98] Nasopharyngeal swabs Laboratory-developed TaqMan PCR methods used as reference	DFA	Influenza A: 62.5 (53.6 to 70.7) Influenza B: 69.7 (52.5 to 82.8)	Influenza A: 100 (98.6 to 100) Influenza B: 100 (98.9 to 100)			
	RT-PCR Simplexa Flu A/B*	Influenza A 2µl sample: 83.3 (75.6 to 89.0) 5µl extract 89.2 (82.3 to 93.7) Influenza B: 2µl sample: 72.7 (55.6 to 85.1) 5µl extract 84.9 (68.6 to 93.8)	Influenza A 2µl sample: 100 (98.6 to 100) 5µl extract: 100 (98.6 to 100) Influenza B: 2µl sample: 100 (98.9 to 100) 5µl extract: 100 (98.9 to 100)			Positive samples with cycle threshold (*CT*) values of_38 were accepted as positive. For *CT* values of >38, amplification was repeated in duplicate and accepted if one replicate was positive. Although Simplexa was less sensitive than current LDT assays, it was simpler, required minimal hands-on time, included an internal control, and had a shorter assay time. Samples missed by Simplexa using extracted samples had very low viral loads. More than 95% of the discrepant results were from adults and, with one exception, were tested late in the course of the illness, when patients presented with secondary complications.

						Thus, authors suggested that the clinical impact of missing low-viral-load samples might be minimal and that a higher detection rate with Simplexa is anticipated in settings where samples from patients presenting early in illness are tested.
Leonardi 2010 [99] assay performance and nasopharyngeal swab	RIDT (EZ Flu)	66.7	100			Detection of novel 2009 influenza A (H1N1)
	Rapid shell vial culture	100	100			
	Traditional tube culture	100	100			
	DFA	80	100			
Pollock 2009 [83] nasopharyngeal swabs or aspiration RT-PCR used as reference	DFA	93	97	95	96	Specimens collected only from symptomatic HCW or patients who met CDC criteria for influenza-like illness, as part of their routine clinical evaluation.
Reina 2010 [100] nasopharyngeal swabs RT-PCR used as reference	EIA	52.9	100	100	79.7	
	Viral culture	94.1	100	100	96.9	

Scheuller 2015 [82] nasal swab, throat swab, or nasal wash	RT-PCR	93 (all) 92 (BMT) 95 (Non-BMT)				The goal of the study was to better understand how influenza diagnostic tests perform in the basic military trainees (BMT) population, and how this performance differs from the general population (Non-BMT)
	EIA	57 (all) 51 (BMT) 60 (Non-BMT)				
	Viral culture	51 (all) 63 (BMT) 41 (Non-BMT)				
Tanei 2013 [89]	RIDT	72.9 (95 CI 61.5 to 84.2)	91.3 (79.7 to 102.8)	95.6 (89.5 to 101.6)	56.8 (40.8 to 72.7)	
Tuuminen 2013 [101] nasopharyngeal aspirates and swab samples DFA as the primary reference method	RIDT (mariPOC)	85.7 (69.7-95.2) (aspirates) 77.3 (54.6-92.2) (swabs) Both aspirates and swabs can be analysed with the mariPOC, although aspirates yielded better sensitivity than swabs.	100 (aspirates) 98.3 (swabs)			The rapid and automated test system mariPOC is based on two-photon excitation fluorometry and the concentrations of antigens and fluorescent tracer on microspheres by antigen-antibody reactions. The technology utilizes microvolume reaction chambers and separation-free fluorescence measurement; it allows real-time follow-up of reaction kinetics, and in this application, the test results are read at approximately 20 min and 2 h from the beginning of the reactions. Strong positive samples can be revealed very rapidly and even the lowest positive samples can be detected at the point of care.
Uyeki 2009 [102] Nasal swab confirmatory	RIDT	27 median 19–32 range	97 median 96–99.6 range	87.5 median 80.0–90.9 range	69.4 median 62.5–79.1 range	

influenza testing by RT-PCR (all sites) and viral culture (sites 1 and 2) for all specimens tested at each site throughout the study period						
WHO. 2005 [103]	RIDT	70-75 median	90-95 Median			
Viral culture as gold standard	IF	70-100 range	80-100 Range	85-94 range	96-100 range	
Zhang 2104 [93]	ELISA	Influenza A 90.7 (95% CI 86 to 96) Influenza B 81.8 (95% CI 61 to 100)	Influenza A 100 Influenza B: 100			

DFA: Direct florescent antibody testing; EIA enzyme immunoassay; ELISA: Enzyme Linked Immunoabsorant Assay; HAI: haemagglutination inhibition assay; IF: immunofluorescence; RIDT: Rapid influenza diagnostic test;

Table 6.2. Ranges of sensitivity, specificity, PPV and NPV levels per each test (Source: results from studies listed in Table 1)

TEST	Range SE (%)	Range SP (%)	Range PPV (%)	Range NPV (%)
DFA	47.2-93	97-100	90.6-100	82.2-96.2
EIA	52.9-60	100	100	79.7
ELISA	81.8-90.7	100		
IF	70-100	80-100	85-94	86.7-100
RIDT	18.2-85.7	90-100	76.5-100	56.8-94.5
RT-PCR	72.7-100	91.1-100	84.7-94.3	99-100
Viral culture	45.7-100	99.8-100	95.5-100	94.8-100

DFA: Direct florescent antibody testing; EIA enzyme immunoassay; ELISA: Enzyme Linked Immunoabsorant Assay; HAI: haemagglutination inhibition assay; IF: immunofluorescence; RIDT: Rapid influenza diagnostic test;

6.3 Recommendations

Vaccine effectiveness studies require the identification of viruses. This should be carried out with sensitive and specific techniques, and provide detailed relevant biological information about the causative agent to avoid confounders. Consequently, this detection should use the most up-to-date diagnostic tools.

The clinical network involved in the DRIVE studies should be able to collect nasal and/or nasopharyngeal swabs as early as possible after symptom onsets and no longer than 7 days after symptoms manifestation (as reflected in D7.1, the protocol for case-control studies), and send these specimens to a corresponding virological lab.

Since evidence shows that virus shedding is significantly reduced already three or four days after disease onset, information regarding the delay between disease onset and the collection of the specimen should be collected in studies with primary data collection and IVE estimates should be stratified according to this delay.

For studies performing primary data collection, we suggest to use labs that:

- Are able to detect influenza by RT-PCR (first line of screening), even if DRIVE will investigate the value of other methods of influenza virus detection in innovative study designs.
- Further characterize the detected virus by sub-typing (for Influenza A viruses) and lineage determination (Influenza B viruses).
- Have their performance assessed by participation in External Quality Assessment (EQA), as those provided by Quality Control for Molecular Diagnostics (QCMD) [104].

If possible, the lab should also be in capacity to carry out additional influenza testing:

- Genotyping of the virus (HA and NA gene sequencing, by Sanger or NGS, for genetic clade determination, full genome sequencing should also be an objective). This can be very helpful for the comparison of strains and interpretation of IVE results.
- Strain characterization for the identification of potential antigenic variants. This means being able to grow influenza viruses on MDCK cells, and subsequently determine their antigenic profile with ferret sera. This will allow the complete antigenic characterization of the influenza viruses, according to the WHO standards as described by the CDC [95].

In addition, the labs may detect with the same techniques other respiratory viruses such as RSV, rhinoviruses, human metapneumoviruses, adenoviruses and parainfluenza viruses; all these viruses can co-circulate during the influenza epidemic and may be responsible for ILI presentation.

Not all labs have all these capacities; if necessary, labs may link with others that can perform these additional tests. FISABIO and UCBL labs can support the implementation of complementary techniques.

For studies using secondary data collection, the preferred lab method to test for influenza is RT-PCR.

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7 Methods for rapid assessment of IVE

7.1 Purpose of rapid IVE assessment

Due to the continuous evolution of the influenza viruses and potential mismatches between the circulating and the vaccine strains [105], the effectiveness of different seasons' influenza vaccines can differ a lot [2]. Rapid or real-time assessment of IVE before the end of the epidemic is important for several reasons including among others to contribute to the monitoring of the benefit-risk assessment of newly reformulated influenza vaccines without undue delay [19].

In case early estimates during a given season are available indicating a low IVE, additional preventive measures can be put into practice to ensure a high level of protection in the population, e.g. through recommendations to continue the use of vaccination, complementary for the use of antivirals to mitigate the influenza associated complications. Likewise, early knowledge about a highly effective vaccine might further increase the vaccine uptake in the population. In either way, rapidly available impact measures communicated to the population already during an epidemic can strengthen individual health and public health. At the same time, rapid or real-time IVE figures calculated before the end of the epidemic may be misleading for the public opinion if not accompanied with appropriate guidances and precautionary measures. An influenza epidemic is often characterised by more than one wave caused by different viruses circulating in different periods. The vaccines' effectiveness might differ between those waves and thus an estimate based on the first wave is not necessarily a good estimate for the rest of the season.

Moreover, early IVE estimates can also benefit future vaccine compositions for the other hemisphere. The WHO regularly reselects the influenza strains to be included in the vaccines [16]. The recommendation for the Northern Hemisphere is usually made by the end of February [103], which consequently marks the time point when the results of the rapid IVE assessment would be expected, at the latest.

7.2 Applied methods for rapid IVE assessment

The estimation of the IVE during an ongoing influenza epidemic lays high demands on the study design, data sources, and the data collection process. However, the only major difference between end-season and rapid (mid-season) IVE assessment in terms of data analysis is the length of the study period. By analysing only those data collected until a respective cut-off day before the end of the influenza epidemic, several studies have recently been published presenting ‘early’, ‘interim’, or ‘mid-season’ estimates [8, 106-111]. The majority of these studies applied the test-negative design [8, 106, 107, 109-111], while only one utilised the cohort design [108]. In the context of rapid IVE assessment, the pros and cons of the two approaches remain as described in 2.3.1 and 2.1 and one

would expect no difference between the study designs as long as the different sources of bias are correctly controlled for (see 5.4).

The challenges of such studies are related to the rapidness in which they must be conducted, and to statistical power considerations. While respiratory specimens should always be analysed in a timely manner in order to benefit a patient's treatment, data collection and statistical analysis do only need to be accelerated for a rapid IVE assessment. Accordingly, there is no time for extensive data validation steps. Moreover, the data must be collected in real-time or with a known delay to ensure that the study period is completely covered by the data on the day of analysis. Because the study period is ended before all cases of an influenza epidemic have occurred, many rapid IVE estimations are characterised by the lack of statistical power due to small numbers of cases. Generally, it is considered that the longer the study period, the more precise the estimates.

This trade-off between rapidness and statistical power and thus reliability of estimates therefore also affects the decision when to conduct IVE studies. All the studies from the Northern hemisphere referenced above had finished their analyses in the first half of February 2017 [8, 106-110], i.e. before the WHO Consultation and Information Meeting on the Composition of Influenza Virus Vaccines for Use in the 2017-2018 Influenza Season [112]. Furthermore, three studies indicated that they had ended the study period about two weeks after the peak of the epidemic curve [108, 109, 111].

7.3 Recommendations for future near real-time IVE assessment

In future, any study design that has been proven to yield valid and reliable estimates can be chosen to rapidly assess IVE in near real-time. Large test-negative case-control studies implemented on top of multiple, already existing influenza surveillance/ sentinel systems and large cohort studies based on automated (secondary) data collections might be the most feasible strategies. The appropriate timing within the season depends on the actual purpose of the IVE figures and the course of the epidemic. It seems advisable to assess IVE early but after the peak of the influenza-type specific epidemic curve, since it has been shown that such studies can reliably predict even end-season IVE [113, 114]. However, in order to provide data to the WHO to support the decision on future vaccine compositions, one might also preliminary end the study period shortly before or at the top of an intense epidemic that started late.

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8 Data analysis for individual studies

8.1 Vaccine effectiveness measures

The effect measures to be estimated and quantified are brand-specific IVE. Influenza vaccine effectiveness is usually defined as

$$VE = 1 - RR,$$

where *RR* denotes the relative risk of the outcome for vaccinated individuals versus unvaccinated individuals [115]. *VE* is estimated by either one minus a confounder-adjusted estimator of the ratio of the influenza attack rates in a cohort, or by one minus a confounder-adjusted estimator of the ratio of the influenza incidence rates in a cohort or a dynamic population. When the study is a case-control design, both the attack rate ratio and the incidence rate ratio are estimated by odds ratios. If the study is a cohort design, the attack rate ratio is estimated by a risk ratio, and the incidence rate ratio by a hazard ratio.

In Table 8.1, for a number of study designs in Chapter 2 the estimator type and the estimator are given.

Table 8.1: Estimator for the analysis of IVE data, by study design and outcome data

Source population	Study design	Type of sampling	Outcome data	Estimator
Cohort	Cohort design		Count data (cases, non-cases)	Attack rate ratio
			Time-to-event data	Hazard ratio
	Nested case-control design	Cumulative sampling	Count data (cases, controls)	Odds ratio
		Density sampling	Count data (cases, controls)	Odds ratio
	Case-cohort	Case-base sampling	Count data (cases, referents)	Attack rate ratio with pseudo-denominators [116]
Dynamic population	Case-control design with density sampling	Density sampling	Count data (cases, controls)	Odds ratio
	Test-negative design	No sampling	Count data (cases, controls)	Odds ratio

The effect measure being estimated in the analysis of influenza vaccine effectiveness data is not *VE*, but the relative risk *RR*. If LL_{RR} and UL_{RR} are the lower and the upper limit of a confidence interval for *RR*, then 1- UL_{RR} and 1-LL_{RR} are the lower and the upper limit for a confidence interval for *VE.*

8.2 Power and sample size

Please refer to WP2 task 5, where sample size estimations for estimating overall and brand-specific IVE using the cohort or case-control design have been produced.

8.3 Adjusting for confounders

8.3.1 Statistical methods

In the statistical analysis confounders can be adjusted for (controlled for) either by stratification or by regression. Stratified analysis works best if the number of confounders to adjust for is small. For each possible combination of confounder levels, a separate stratum must be created, with the risk of a large amount of sparsely populated strata with too little data to estimate the association between vaccination and prevention of the outcome with any reasonable degree of precision. Given that in VE studies the number of confounders to be adjusted for is usually non-small, stratification to adjust for confounding is not advised. On the other hand, stratification is an important tool to inspect effect modification, see Section 8.4.

Alternative approaches for confounder adjustment are regression and propensity scoring.

8.3.2 Regression models

Confounders are adjusted for by including them as covariates in the regression model.
In Table 8.2 appropriate regression models for the analysis of influenza vaccine effectiveness data are listed by source population, study design and type of data.

Table 8.2: Regression models for the analysis of IVE data, by study design and type of outcome data

Source population	Study design	Outcome data	Regression model
Cohort	Cohort design	Count data (cases, non-cases)	General log-linked binomial regression model Poisson regression
		Time-to-event data	Cox regression
	Nested case-control design	Count data (cases, controls)	Unconditional logistic regression
	Case-cohort design	Count data (cases, controls)	Unconditional logistic regression with pseudo-likelihood [117]
Dynamic population	Case-control design with density sampling	Count data (cases, controls)	Conditional logistic regression with matching on calendar time
	Test negative design	(cases, controls)	Conditional logistic regression with matching on calendar time

Logistic regression for nested case-control designs with cumulative sampling

Logistic regression is the standard regression model for the analysis of nested case-control data. The parameter of interest is the odds ratio (*OR*). If the influenza attack rates in the cohort are low, *OR* ≈ *RR*.

Logistic regression for case-control designs with density sampling

Just as in logistic regression for nested case-controls designs, in logistic regression for case-control designs with density sampling the input data are numbers of cases and controls. However, there are some subtle differences to be aware of:

- the parameter being estimated is the odds ratio, but in this design *OR = incidence density ratio (IDR)*
- controls represent person-time, individuals may be at different times sampled more than once as control, or may become a case after being sampled as a control; these should be treated as independent observations [118]
- a person who has been sampled twice at different times, may have changed his/her vaccination status in between
- to control for seasonal patterns or time-dependent differences in the vaccinated/unvaccinated ratio, calendar time should be included in the regression model

Cox regression

The Cox regression model is widely used for the analysis of time-to-event data. The parameter of interest is the hazard ratio *HR*, which can be interpreted as the influenza incidence rate ratio. The time-to-event is defined as the time-span between the start of the influenza season and the occurrence of the outcome, assuming that all vaccinations were given prior to the start of the influenza season, or alternatively vaccination can be modelled as a time-varying variable. For subjects who did not experience an event, the time-to-event is right-censored at death, moving out of the catchment areas, receiving influenza vaccination other than the defined exposure (when studying type or brand-specific IVE), having (confirmed) influenza infection other than defined outcome (only applicable for influenza type- or strain-specific analysis), or the study period, whichever comes first.

8.3.3 Representing confounders in the regression model

The approach advised to represent categorical (dichotomous, nominal and ordinal) confounders in the regression model is using indicator variates, because this approach maximizes the thoroughness of control [119]. If a categorical confounder has *k* categories, then (*k*-1) indicator variates must be defined:

X_2 = indicator for the second category

.

.

X_k = indicator for the k^{th} category

The first category is the reference category.

Incorrect modelling of a continuous confounder can result in residual confounding. If the association between the continuous confounder and risk of influenza infection is not linear, but, for example, U- or J-shaped, the assumption of a linear relation between the confounder and influenza infection can result in substantial residual confounding. Two sophisticated but challenging to interpret methods to model the relation between a continuous variable and an outcome are fractional polynomials [120] and restricted cubic splines [121]. However, it has been shown that adjustment for a continuous confounder by means of the much simpler method of stratification of the confounder in 5 strata, and use of fractional polynomials or restricted cubic splines yield similar results [122]. Therefore, stratification to adjust for continuous confounders should be the preferred approach.

8.3.4 Selecting confounders in the regression model

For confounder selection the following strategy is advised:

- all *known* confounders specified in the protocol (dictated by the knowledge of the disease, the medical understanding) should be adjusted for, that is, be included ("forced") in the regression model, regardless of their significance in the specific study.
- select possible confounders stepwise, one by one in the model, based on the change-in-estimate criterion, that is, at each step add that confounder that leads to the greatest change in the estimate of the relative risk
- stop adding variables to the model if the changes in the relative risk estimate become non-meaningful; popular choices for cut-offs for non-meaningful changes are 5% and 10%

An alternative to the forward-selection strategy is the backward-deletion strategy. The reason why the forward-selection strategy is advised is that the backward-deletion strategy cannot be implemented when the problem of sparse data (see below) occurs.

When applying this, one should be aware of the risk of sparse data bias. This bias occurs when for some combinations of confounders there are no or only a few infected cases, and it can occur even in quite large data sets. The symptom of sparse data bias is that the relative risk estimates get smaller

and smaller, away from seeing no effect (that is, from 1.0), often with excessively wide confidence intervals. This should not be interpreted as evidence of confounding. A rule of thumb is that *confounder-adjusted influenza vaccine effectiveness estimates often only differ modestly from the unadjusted estimate*. If sparse data bias is suspected, reducing the number of strata may be attempted. A diagnostic test for the risk of sparse data bias is that the total number of cases divided by the total number of variables in the model should not be lower than 7.

A consequence of sparse data bias may be that not all confounders can be controlled for, in which case propensity scoring should be attempted.

8.3.5 Propensity score method

The propensity score method removes confounding caused by the observed covariates, by balancing baseline covariates values between vaccinated and unvaccinated subjects [123]. This is achieved by assigning each subject a so-called 'propensity score.' The propensity score is then the predicted probability of being vaccinated. VE estimates are obtained by adjusting for the propensity score as a linear or categorical variable or by matching subjects with similar propensity scores. While in many cases similar results will be obtained, there are important potential advantages to propensity scoring over conventional regression. For example, with propensity scoring one need not to be concerned with overparameterization and can include non-linear terms and interactions. When influenza attack rates are low but vaccination is common, propensity scoring may be a better option than logistic regression if many confounders must be adjusted for. Finally, propensity scoring tends to the more robust method. For an example of a vaccine effectiveness with propensity scoring see, Simpson et al. [124].

8.4 Effect modification

An effect modifier is a variable that differentially (positively or negatively) modifies the observed effect of the exposure on the outcome. Different groups have different risk estimates when effect modification is present [42]. A known effect modifiers is age. The standard approach to study effect modification is to divide the effect modifier in two or more distinct strata, add the appropriate product (interaction) terms (vaccination times stratum) to the statistical model.

8.5 Missing covariate data

Missing covariate data can be handled by multiple imputing (MI) [125, 126]. Simulation results suggest the application of predictive mean matching after regression switching is the optimal approach, unless 50% or more of the subjects have missing data, or the missing data are 'missing not at random'

(MNAR) [127]. When data are MNAR, a pattern-mixture model (PMM) approach is advised. For a discussion of this approach as well as publicly available SAS macros, see chapter 7 of the book by O'Kelly and Ratitch [128].

8.6 Statistical models to deal with limitations of laboratory tests

In order to adjust for non-differential disease misclassification, correction equations, such as that showed in Figure 8.1, (in which π_{Other} is the risk of disease due to other pathogens than those targeted by the vaccine, p_0 the observed disease prevalence among the subjects indicated as unvaccinated, p_1 the observed prevalence among the subjects indicated as vaccinated, and SP_d the disease specificity of the case definition), which requires the disease specificity estimate, should be applied to the estimate of vaccine effectiveness [129].

$$VE_{\pi} = 1 - \frac{p_1 - (1 - SP_d)\pi_{Other}}{p_0 - (1 - SP_d)\pi_{Other}}.$$

Figure 8.1 Correction equation of IVE adjusting for disease misclassification. Source: [129]

8.7 Recommendations

When analysing data of an individual VE study, the design of the study should be taken into account, because the parameter that can be used to estimate the VE depends differs between designs. Confounders of the relationship between vaccination and prevention of infection should be adjusted for, which can be achieved by regression or propensity scoring. Known confounders should be included in the statistical model, regardless of their significance. Other – potential – confounders, should be selected in the model by forward-selection. In case of sparse data, it may not be possible to control for all confounders, in which case propensity scoring may be attempted.

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9 Pooling data from different data sources

9.1 Prerequisites

Brand specific vaccine effectiveness is expected with near certainty to need pooling of data across studies. Differences between studies should be limited where possible to allow pooling. The following aspects should be taken into account:

- Limit difference between individual study designs by:
  - Reducing choices in local study design
  - Clear definitions and optimal compliance
- Pool data across settings with similar characteristics (i.e. primary care vs hospital setting)
- Define upfront the minimum framework to pool the data
- Define quality criteria
- Align local designs by using generic protocol to ensure homogeneity
  - Define common screening/eligibility criteria used to identify cases/controls (Reasons for GP consultations/hospital admission)
- Define a minimum set of data to be collected

9.2 One-stage vs. two-stage pooling

There are two statistical approaches for pooling data: a one-stage or a two-stage pooling approach. The two-stage approach refers to the classical meta-analytical approach, also called aggregated data meta-analysis (AD-MA). In this approach, the patient-level or minimally aggregated data from each study are analysed separately in order to obtain the effect estimates of interest (here vaccine effectiveness) and the corresponding confidence intervals (CIs). Then, in the second step, the effect estimates are combined by an appropriate meta-analysis model to obtain the meta-analytical (pooled) estimate. The one-stage pooling approach analyses all the combined patient-level or minimally aggregated data from the different data sources in a single step. This approach is also called the individual participant data meta-analysis (IPD-MA).

9.2.1.1 Two-stage pooling

Two-stage pooling or AD-MA is the mainstay of systematic reviews [130]. This is the ‘classical’ meta-regression approach, by which aggregated data (typically effect measures) are combined into a pooled estimate and heterogeneity is quantified and possibly explained. There are two popular statistical models for AD-MA, the fixed effect model and the random effects model [131]. A fixed effect meta-analysis assumes all studies are ‘replicates’ estimating exactly the same effect with the differences between study estimates solely explained by sampling variability. The random effects

meta-analysis assumes the observed estimates can vary across studies because of 'true' effect differences across populations, differences in the conduct of the study etcetera on top of sampling variability. Heterogeneity between studies is typically quantified and the sources of heterogeneity can be explored using meta-regression or stratified analysis.

If studies are comparable in terms of population, exposure and case definitions and control for confounding, pooling is considered appropriate irrespective of study design. Several examples of two-stage pooling of IVE estimates, including estimates from studies with different study designs, exist [132, 133].

9.2.1.2 One-stage pooling

The application of one-stage pooling or IPD-MA has increased over the last decade, with many examples of combining clinical trial data, particularly in the area of cancer and cardiovascular disease interventions. In 2015, PRISMA-IPD guidelines for reporting systematic reviews and meta-analyses of IPD were published [134]. Advantages of IPD-MA compared to literature-based AD-MA include checking and transforming data to common sources or measures, standardizing analysis and increased flexibility in performing statistical analyses and common reporting. Major disadvantages of IPD-MA include being very time- and resource-intensive and requiring high levels of (international) collaboration [130], furthermore data protection standards may restrict the feasibility of this type of analysis. Random effects (multilevel) regression models are used to jointly analyse IPD from all studies while accounting for the (within-study) clustering of subjects [135]. Different model specifications are possible, including correlated and independent random effects as well as stratified random-effects models [136].

9.3 Equivalence of one-stage and two-stage pooling

The one-stage and two-stage approach have been shown to be equivalent in many situations based on theoretical considerations [137-139], simulations [136] or empirical comparisons [140] provided exactly the same data was used. However, some studies have shown different results for both approaches in some occasions [136]. Burke et al explain why there might be differences [141]. The reasons relate to different modelling assumptions, parameter specifications and estimation methods, which might sometimes be subtle. In case of small number of studies or small number of events, the IPD-MA may be preferred in order to be able to use exact likelihood methods and avoid having to make incorrect assumptions about the between-study variance [141]. However, usually the AD-MA will suffice [140].

9.4 Combining both approaches

Subject-level data are not always available for all relevant studies and individual-level and aggregated data needs to be combined. There are three approaches for combining IPD and AD in a meta-analysis [142]. First, the available IPD is reduced to AD and then pooled with the other AD. Second, it might be possible to construct IPD from published aggregate information (based on 2x2 tables), which is subsequently combined with the other IPD for analysis. Third, in hierarchical-related regression, the IPD and AD data are analysed jointly.

9.5 Recommendation

The objective of DRIVE is to estimate brand-specific influenza vaccine effectiveness in Europe by combining data from different study sites. Some site-specific studies will adopt the test-negative case-control study design while others will use a cohort design. These different study designs imply that different data will be collected and that different statistical analyses are needed to analyse these data (i.e. logistic or conditional logistic regression for case-control versus Poisson or Cox regression for cohort studies). Given the statistical equivalence of AD-MA and IPD-MA and given the additional complexity or even impossibility of performing IPD-MA when data are collected using different study designs, the AD-MA is the preferred method for combining data from different study sites. Furthermore, many of the mentioned advantages of IPD-MA (i.e. transforming data to common sources or measures and standardizing analysis) can also be achieved through harmonization/standardization of the individual site-specific studies.

Within AD-MA, the random effects meta-analysis approach is preferred to the fixed effects approach for combining the data from the site-specific studies on influenza vaccine effectiveness. The assumption that the between-study variability is explained by sampling variability only, which underlies the fixed effects meta-analysis, is not realistic for the studies on influenza vaccine effectiveness in Europe. There are many differences between these studies, including differences in population, design, exposure- and disease ascertainment as well as in covariate adjustment. For DRIVE, we recommend a random effects meta-analysis approach for every effect measure of interest, possibly stratifying over effect modifiers, while in the same time standardizing the design and conduct of the site-specific studies to the extent possible.

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10 Summary of recommendations

In this section, the recommendations provided at the end of each chapter are listed.

Study design

For studies using primary data to monitor IVE, we suggest to use the test-negative case-control design, with an appropriate choice of control group and implementation of sampling protocols [15], or alternatively the prospective cohort design.

For studies using secondary data we suggest to use the cohort design.

Exposure

The following data on exposure should be collected, both for studies collecting primary data and those using secondary data:

- Vaccine brand(s) of all influenza vaccinations given during the index season (i.e. the season for which IVE is being estimated)
- Vaccination date(s) of all influenza vaccinations given during the index season, or if not available, the sequential order and relative timings of exposure and outcome
- How the vaccination status was ascertained and whether it was confirmed e.g. through medical records
- For children: how many doses they should have received based on vaccination in previous seasons (i.e. one dose if they have received influenza vaccination in an earlier season, two doses if they have not previously received influenza vaccination) and how many they actually received.

Outcome

In studies collecting primary data, the recommended outcome is influenza test-positive, medically-attended ILI or SARI. We suggest the collection of the following data points:

- Symptoms forming the clinical syndrome of ILI or SARI including the information whether hospitalisation or intensive care treatment was required,
- Date of symptom onset,
- Date the respiratory specimen was taken,

- Detected influenza type and preferably also subtype/ lineage for laboratory confirmed influenza.

In studies utilising secondary data e.g. from existing health care databases, the recommended outcome is laboratory-confirmed influenza, overall or stratified by clinical condition. However, this recommendation does not exclude the use of syndromic or unspecific outcome definitions discussed in 4.1, either in association with a positive influenza test or alone. We suggest the collection of the following data points:

- Clinical condition (if applicable),
- Date the respiratory specimen was taken,
- Detected influenza type and preferably also subtype/ lineage for laboratory confirmed influenza.

Potential biases and confounders

For studies using the test-negative design, we suggest the collection of the following data points:

- Age (stratification by age groups, minimally 6 months-14 years; 15-64 years; 65+ years)
- Gender
- Chronic underlying conditions (e.g. liver disease, heart disease, diabetes, cancer, immunodeficiency/ organ transplant, autoimmune disease, lung disease, anemia, renal disease, dementia, history of stroke, rheumatologic diseases, obesity)
- Use of influenza antivirals (type, timing)
- Lag time between symptom onset and testing

Not all data points that can be collected through primary data collection are available in secondary data. For studies using secondary data, we suggest the collection of the following data points.

- Age (stratification by age groups, minimally 6 months-14 years; 15-64 years; 65+ years)
- Gender
- Chronic underlying conditions
- Past healthcare use (e.g. nr of GP visits, nr of hospitalizations)

Each study's SAP should explain how the data will be used in the analysis. Information on how to adjust for confounders in the analysis can be found in Chapter 8.

Optimization of the value of microbiological and virological information

Vaccine effectiveness studies require the identification of viruses. This should be carried out with sensitive and specific techniques, and provide detailed relevant biological information about the

causative agent to avoid confounders. Consequently, this detection should use the most up-to-date diagnostic tools.

The clinical network involved in the DRIVE studies should be able to collect nasal and/or nasopharyngeal swabs as early as possible after symptom onsets and no longer than 7 days after symptoms manifestation (as reflected in D7.1, the protocol for case-control studies), and send these specimens to a corresponding virological lab.

Since evidence shows that virus shedding is significantly reduced already three or four days after disease onset, information regarding the delay between disease onset and the collection of the specimen should be collected in studies with primary data collection and IVE estimates should be stratified according to this delay.

For studies performing primary data collection, we suggest to use labs that:

- Are able to detect influenza by RT-PCR (first line of screening), even if DRIVE will investigate the value of other methods of influenza virus detection in innovative study designs.
- Further characterize the detected virus by sub-typing (for Influenza A viruses) and lineage determination (Influenza B viruses).
- Have their performance assessed by participation in External Quality Assessment (EQA), as those provided by Quality Control for Molecular Diagnostics (QCMD) [104].

If possible, the lab should also be in capacity to carry out additional influenza testing:

- Genotyping of the virus (HA and NA gene sequencing, by Sanger or NGS, for genetic clade determination, full genome sequencing should also be an objective). This can be very helpful for the comparison of strains and interpretation of IVE results.
- Strain characterization for the identification of potential antigenic variants. This means being able to grow influenza viruses on MDCK cells, and subsequently determine their antigenic profile with ferret sera. This will allow the complete antigenic characterization of the influenza viruses, according to the WHO standards as described by the CDC [95].

In addition, the labs may detect with the same techniques other respiratory viruses such as RSV, rhinoviruses, human metapneumoviruses, adenoviruses and parainfluenza viruses; all these viruses can co-circulate during the influenza epidemic and may be responsible for ILI presentation.

Not all labs have all these capacities; if necessary, labs may link with others that can perform these additional tests. FISABIO and UCBL labs can support the implementation of complementary techniques.

For studies using secondary data collection, the preferred lab method to test for influenza is RT-PCR.

Methods for near-related time assessment of IVE

In future, any study design that has been proven to yield valid and reliable estimates can be chosen to rapidly assess IVE in near real-time. Large test-negative case-control studies implemented on top of multiple, already existing influenza surveillance/ sentinel systems and large cohort studies based on automated (secondary) data collections might be the most feasible strategies. The appropriate timing within the season depends on the actual purpose of the IVE figures and the course of the epidemic. It seems advisable to assess IVE early but after the peak of the influenza-type specific epidemic curve, since it has been shown that such studies can reliably predict even end-season IVE [113, 114]. However, in order to provide data to the WHO to support the decision on future vaccine compositions, one might also preliminary end the study period shortly before or at the top of an intense epidemic that started late.

Data analysis for individual studies

When analysing data of an individual VE study, the design of the study should be taken into account, because the parameter that can be used to estimate the VE depends differs between designs. Confounders of the relationship between vaccination and prevention of infection should be adjusted for, which can be achieved by regression or propensity scoring. Known confounders should be included in the statistical model, regardless of their significance. Other – potential – confounders, should be selected in the model by forward-selection. In case of sparse data, it may not be possible to control for all confounders, in which case propensity scoring may be attempted.

Pooling data from different data sources

The objective of DRIVE is to estimate brand-specific influenza vaccine effectiveness in Europe by combining data from different study sites. Some site-specific studies will adopt the test-negative case-control study design while others will use a cohort design. These different study designs imply that different data will be collected and that different statistical analyses are needed to analyse these data (i.e. logistic or conditional logistic regression for case-control versus Poisson or Cox regression for cohort studies). Given the statistical equivalence of AD-MA and IPD-MA and given the additional complexity or even impossibility of performing IPD-MA when data are collected using different study designs, the AD-MA is the preferred method for combining data from different study sites. Furthermore, many of the mentioned advantages of IPD-MA (i.e. transforming data to common sources or measures and standardizing analysis) can also be achieved through harmonization/standardization of the individual site-specific studies.

Within AD-MA, the random effects meta-analysis approach is preferred to the fixed effects approach for combining the data from the site-specific studies on influenza vaccine effectiveness. The

assumption that the between-study variability is explained by sampling variability only, which underlies the fixed effects meta-analysis, is not realistic for the studies on influenza vaccine effectiveness in Europe. There are many differences between these studies, including differences in population, design, exposure- and disease ascertainment as well as in covariate adjustment. For DRIVE, we recommend a random effects meta-analysis approach for every effect measure of interest, possibly stratifying over effect modifiers, while in the same time standardizing the design and conduct of the site-specific studies to the extent possible.

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