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I-MOVE+

Generic Protocol for the Test Negative Design case control studies to measure pandemic and seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States

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Table of contents

1.	Background	5
2.	Objectives	7
3.	Methods	7
3.1.	Study design	7
3.2.	Study population	8
3.3.	Study period	8
3.3.1.	Seasonal influenza vaccine:	8
3.3.2.	Pandemic vaccine:	8
3.4.	Outcome	8
3.5.	Cases	8
3.5.1.	ILI definition	8
3.5.2.	Influenza case	9
3.5.3.	Laboratory confirmation	9
3.6.	Case finding	9
3.6.1.	Case identification	9
3.6.2.	Case inclusion criteria	10
3.6.3.	Case exclusion criteria	10
3.7.	Controls	10
3.7.1.	Control exclusion criteria	10
3.8.	Exposure (vaccination)	11
3.8.1.	Definition of vaccination status	11
3.8.2.	Vaccination status ascertainment	11
3.9.	Confounding factors and effect modifiers	12
3.9.1.	Chronic diseases	12
3.9.2.	Severity	13
3.9.3.	Smoking history	13
3.9.4.	Previous influenza vaccinations	13
3.9.5.	Pneumococcal vaccination	13
3.9.6.	Functional status	14
3.9.7.	Number of GP consultations in the previous 12 months	14
3.9.8.	Antiviral administration	14
3.9.9.	Source of information	14
3.10.	Sample size	14
3.11.	Data	17
3.11.1.	Collected information	17
3.11.2.	Data validation	18
3.12.	Analysis	18
3.12.1.	Individual study site analysis	18
3.13.	Data management	22
3.13.1.	Individual analysis	22
3.13.2.	Data cleaning	22
3.13.3.	Pooled analysis	22
3.14.	Potential biases	22
3.14.1.	Negative confounding	22
3.14.2.	Positive confounding	23



European Union

- 3.14.3. Representativity of cases 23
- 3.14.4. Pooled estimate and its bias 23
- 3.15. Dissemination of results 23
 - 3.15.1. Publications, scientific communication 23
- 3.16. Training..... 24
- 4. Logistical aspects.....24
 - 4.1. Study leader..... 24
 - 4.2. Human resources..... 24
 - 4.3. Supervision 24
 - 4.4. Questionnaires 24
 - 4.5. Computer support..... 25
 - 4.6. Consent..... 25
 - 4.7. Report..... 25
- Annex 1: List of variables, definitions and coding..... 28
- Annex 2: Genetic and antigenic analysis data (examples) 32
- Annex 3: Pooled data management..... 34
- Annex 4: Pooled data analysis 38
- Annex 5: Data flow for pooled database 43
- Annex 6: Generated/recoded variables 44
- Annex 7: Stata syntax 47
- Annex 8: Measuring VE by time since vaccination 49
- Annex 9: Study-specific annexes..... 53



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Abbreviations

ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EMA	European Medicines Agency
EU	European Union
GP	General Practitioner
ILI	Influenza-like illness
LAIV	Live Attenuated Influenza Vaccine
MS	Member States
OR	Odds ratio
TND	Test negative design
VC	Vaccination coverage
VE	Vaccine effectiveness
<input checked="" type="checkbox"/>	(Tick/check mark indicates the sections that Member States should adapt and detail in their study annexes.)



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1. Background

In 2009 the European Council of Ministers recommended that all EU MS reach an influenza vaccination coverage of 75% in all risk groups by the winter season 2014-15. Risk groups are defined as individuals 60 or 65 years and older, and people with a range of underlying medical conditions¹.

Influenza viruses are the only vaccine preventable viruses that undergo frequent genetic and antigenic changes. Vaccine induced immunity is not known to last beyond 6-12 months, perhaps less. As a consequence, the influenza vaccine is reformulated each year and annual revaccination is recommended. Available seasonal influenza vaccines are only moderately effective and vaccine effectiveness (VE) may vary between vaccines types and products. Observed VE varies from year to year, between population subgroups (age-groups, risk groups) and differs for the various influenza type and subtype outcomes measured.

Influenza VE is only partially correlated to the degree of virological match between the virus strains included in the vaccine and the circulating strains in an influenza season or a pandemic. Immunologic correlates of protection are not well defined. As a consequence, starting in 2014 the European Medicines Agency (EMA) has stopped requiring yearly immunogenicity studies from vaccine producers prior to marketing their products. From 2015, EMA will require product-specific VE data².

With the exception of some of the 2009 pandemic vaccines and of some new vaccine formulations (LAIV), all the seasonal trivalent and more recently quadrivalent influenza vaccines are authorised nationally and their evaluation is so far outside of the EMA remit. The available vaccine products, the target groups for vaccination and the vaccination coverage vary across countries. New vaccines are being developed for which limited or no effectiveness data are yet available in the EU. Several studies suggest that adjuvanted vaccines are more immunogenic against seasonal influenza than non-adjuvanted in the elderly population but their protective effect against clinical disease is unclear. A comparison of adjuvanted and non-adjuvanted vaccines would provide essential information for vaccine recommendations and health economic assessments.

Lack of early in the season VE by product and influenza type/subtype may result in inappropriate or delayed provision of the most effective vaccine and failure to use alternative measures (antivirals as a preventive measure in case of low VE estimates), increased disease burden and increased costs. I-MOVE+ will provide early and final influenza VE estimates in the elderly population to the WHO to complement the virological information used to select the strains included in the vaccines.

¹ European Commission. Proposal for a Council recommendation on seasonal influenza vaccination. COM(2009) 353/final/2, (2009) http://ec.europa.eu/health/ph_threats/com/Influenza/docs/seasonflu_rec2009_en.pdf

² European Medicines Agency. Guideline on influenza vaccines.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/07/WC500170300.pdf [Accessed May 2015]



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Several questions have recently been raised and the answers to these may modify our understanding of influenza immunology, the vaccines needed to prevent influenza and the recommended strategies required.

The first question is to understand why the measured VE decreases during some of the influenza seasons^{3,4,5,6}. Among potential explanations are the respective role of mutations of circulating viruses during the season and the potential decreasing protection conferred by seasonal vaccines given from October each season. The second question is to understand if and how former seasonal influenza vaccinations modify the effectiveness of current seasonal vaccines^{7,8}. Long term multicentre studies allowing for the early and late season measurement of influenza vaccine effectiveness and with a sufficient sample size to respond to these questions are needed.

In case of an influenza pandemic, an established EU platform to rapidly measure influenza VE by vaccine type and product will allow the evaluation of any pandemic vaccine and the adaptation of preventive and control strategies.

I-MOVE (Influenza Monitoring Vaccine Effectiveness in Europe), the first network to monitor influenza vaccine effectiveness within and across the seasons in the EU and the European Economic Area (EEA) was established in 2007⁹. The network was funded by the European Centre for Disease Prevention and Control (ECDC) and MS. It is coordinated by EpiConcept (a Small and Medium Enterprise) and includes public health institutes from the EU and EEA. Building on this network, the I-MOVE+ platform will increase the number of participating sites to achieve sufficient sample sizes for the study questions listed above. In contrast to I-MOVE, I-MOVE+ targets only the elderly, as they are one of the main target groups for influenza vaccine across Europe. They also provide unique features in relation to burden of disease and immunosenescence. We will include 15 study sites involving 2000 GPs from sentinel networks in EU MS. The laboratory component of the network will include regional and national reference centres from the participating countries. The integration of the virological data will be essential to interpret vaccine effectiveness and impact of the vaccination programmes, to define how laboratory indicators of vaccine-agent and actual vaccine field performance relate, and to trigger further investigations if they diverge.

³ Kissling E, Valenciano M, Larrauri A, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case-control study. *Eurosurveillance* 2013 Jan 31;18(5)

⁴ Pebody RG, Andrews N, McMenamin J, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. *Eurosurveillance* 2013 Jan 31;18(5)

⁵ Castilla J, Martinez-Baz I, Martinez-Artola V, et al. Decline in influenza vaccine effectiveness with time after vaccination, Navarre, Spain, season 2011/12. *Eurosurveillance* 2013 Jan 31;18(5)

⁶ Skowronski DM, Janjua NZ, Sabaiduc S, De Serres G, Winter AL, Gubbay JB, et al. Influenza A/subtype and B/lineage effectiveness estimates for the 2011-12 trivalent vaccine: cross-season and cross-lineage protection with unchanged vaccine. *J Infect Dis.* 2014; Jan 19.

⁷ Ohmit SE, Thompson MG, Petrie JG, Thaker SN, Jackson ML, Belongia EA, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. *Clin Infect Dis.* 13 nov 2013

⁸ Sullivan SG, Kelly H. Comments on: Influenza Vaccine Effectiveness in the Community and the Household. *Clin Infect Dis.* mai 2013;56(10):1363-9.

⁹ Valenciano M, Ciancio BC, on behalf of the I-MOVE study team. I-MOVE a European network to measure the effectiveness of influenza vaccines. *Euro Surveilance.* 2012;17(39):pii=20281.



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While each of the study sites can be analysed separately, pooling them into one analysis will provide a sample size big enough to answer study questions with a reasonable precision.

The study sites will carry out case control studies, based on the test negative design, which is the main design used in influenza VE studies and is recommended by EMA^{10,11,12}.

This publication presents the core European protocol for the GP-based study component of I-MOVE+, outlining the agreed methods for measuring pandemic and seasonal VE for each of the individual studies. The protocol includes a plan for the pooled analysis. The specificities of each study can be detailed in the study annexes. The protocol will be updated according to the final vaccination strategy (target groups, vaccine delivery, vaccine products available and number of doses) in each of the participating MS, the time when the vaccine will be available, the extent of the virus circulation and the identification of new groups at risk.

2. Objectives

To measure in the elderly population (aged ≥ 65 years) at primary care level, early and late in the season, the direct effect (effectiveness) of various types of influenza vaccines against laboratory confirmed (PCR positive) influenza for each study site and in a pooled analysis in order to:

1. Identify vaccine types (e.g. adjuvanted vs. non-adjuvanted, groups of vaccines (split virion, subunit, adjuvanted, trivalent vs. quadrivalent.)) and products with different effectiveness,
2. Understand the factors affecting influenza vaccine effectiveness (VE), the duration of protection, the role of repeated seasonal vaccinations,
3. Identify key influenza virus phenotypic or genotypic evolutions that could affect vaccine performances and estimate VE against specific clades.

3. Methods

3.1. Study design

- Test negative design case control study in each participating country.
- Multicentre case control study using data from several countries.

¹⁰ Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. *Vaccine*. 2013 Apr 19;31(17):2165-8. doi: 10.1016/j.vaccine.2013.02.053.

¹¹ Valenciano M, Ciancio BC, on behalf of the I-MOVE study team. I-MOVE a European network to measure the effectiveness of influenza vaccines. *Euro Surveill*. 2012;17(39):pii=20281.

¹² European Medicines Agency. Guideline on Influenza Vaccines.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/07/WC500170300.pdf [accessed May 2015]



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3.2. Study population

The study population is community-dwelling individuals aged 65 years and above with no contra-indication for influenza vaccination who consult a participating GP if they develop ILI.

3.3. Study period

The study period starts when the influenza virus is circulating and the influenza vaccine is available.

3.3.1. Seasonal influenza vaccine:

The study period starts at the beginning of the seasonal influenza period and >14 days after the start of the influenza vaccination campaign and finishes at the end of the influenza period.

- Inclusion period: Cases and controls are included from the week of onset of the first influenza positive case included in the study.
- Each study defines the beginning, the peak and the end of the study period according to the information provided by the country influenza sentinel surveillance system (details available in the study annexes).
- Each study specifies the date of the start of their vaccination campaign.

3.3.2. Pandemic vaccine:

the study period is defined depending on the gradual availability of vaccines and the pandemic incidence.

- Each study defines the beginning and end of the pandemic VE study period.

3.4. Outcome

The outcomes of interest are:

- subtype-specific laboratory-confirmed influenza A,
- laboratory-confirmed influenza B overall and if available by lineage (B Victoria/B Yamagata),
- laboratory-confirmed influenza by clade (where possible).

3.5. Cases

3.5.1. ILI definition

A case of influenza like illness (ILI) is defined as an individual who consults a participating GP, presenting a sudden onset of symptoms AND at least one of the following four systemic symptoms:

- Fever or feverishness
- Malaise
- Headache
- Myalgia

AND at least one of the following three respiratory symptoms:



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- Cough
- Sore throat
- Shortness of breath

For the pandemic vaccine, the ILI case definition may be revised during the course of the pandemic.

3.5.2. *Influenza case*

An influenza case is defined as an ILI case with a respiratory sample positive for influenza with at least type/subtype information.

- Indicators to define cases are specified in the study annexes.

3.5.3. *Laboratory confirmation*

Specimens are collected from ILI cases ≥ 65 years who consult their GP within seven days of symptom onset.

- Mode of specimen collection, storage and transport for each study are listed in the study annexes.

Influenza laboratory confirmation is provided by using RT-PCR.

- RT-PCR characteristics for each study are listed in the study annexes.

Isolates undergo a molecular analysis for currently circulating influenza A viruses (subtypes H3 and H1) and influenza B.

Following the procedures outlined by each study, a systematic sample of isolates (or all isolates) undergo gene sequencing. The sampling procedure can include sequencing isolates of all elderly, or a systematic sample thereof. The systematic sample should be representative of cases and be large enough to provide reasonable precision when calculating proportions of virus change over time.

- The selection of isolates for each study is specified in the study annexes.
- Each study site is to specify laboratory procedures for genetic and antigenic tests.

3.6. Case finding

3.6.1. *Case identification*

Cases are identified among patients presenting to a participating GP with ILI.

Following the procedures outlined by each study, all ILI cases (if sampling all ILI cases is preferred, but if not possible, then a systematic sample can be taken, e.g. the first 2 ILI cases seen each week per GP) are selected and asked to provide a nasal/throat swab specimen for influenza testing. Influenza-positive ILI cases are considered as influenza cases.

- Description of the GPs participating in each of the studies (number, distribution, catchment population) is available in the study annexes.
- Description of procedures to select ILI cases to swab is available in the study annexes



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3.6.2. *Case inclusion criteria*

Cases are eligible if they meet the above case definition and accept to participate.

- Oral informed consent or written informed consent according to country procedures, as specified in the study annexes.

3.6.3. *Case exclusion criteria*

Cases are excluded if they:

- refuse to participate in the study;
- are not eligible for influenza vaccination due to a condition listed in the summary of product characteristics;
- are institutionalised;
- are unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons;
- are swabbed >7 days after symptom onset;
- are <65 years of age;
- have received antivirals prior to swabbing;
- had tested positive before to any influenza virus in the current season.

Reasons for exclusion are documented.

3.7. Controls

Controls are ILI cases that tested negative for influenza.

3.7.1. *Control exclusion criteria*

Controls are excluded if they:

- refuse to participate in the study;
- are not eligible for influenza vaccination due to a condition listed in the summary of product characteristics;
- are institutionalised;
- are unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons;
- are swabbed >7 days after symptom onset;
- are <65 years of age;
- have received antivirals prior to swabbing;
- tested positive to any influenza virus in the current season.

Reasons for exclusion are documented.



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3.8. Exposure (vaccination)

3.8.1. Definition of vaccination status

Current seasonal influenza vaccine:

- an individual is considered as vaccinated against influenza if the vaccination occurred more than 14 days before disease onset.
- an individual is considered as unvaccinated if they did not receive influenza vaccine in the current season or if they were vaccinated ≤ 14 days before symptom onset.

Product specific seasonal influenza vaccine:

An individual is considered as vaccinated against influenza with a product specific vaccine if he/she has received a vaccination with an influenza vaccine of a named product (see section 3.8.2) more than 14 days before disease onset

an individual is considered as unvaccinated if they did not receive influenza vaccine in the current season or if they were vaccinated ≤ 14 days before symptom onset.

Pandemic vaccine:

- The definition of vaccinated, partially vaccinated and unvaccinated will be defined when it is known how many doses of vaccine are recommended. Once this is known the protocols will be updated.

3.8.2. Vaccination status ascertainment

The exposure of interest in this study is a vaccination history with trivalent/quadrivalent influenza vaccine (for seasonal vaccine) and vaccination history with the pandemic vaccine (in case of a pandemic). The vaccination history includes date of administration and product names. Documenting the flu batch codes (where this is feasible) will allow identifying the vaccine product, the vaccine content (seasonal, pandemic) and the dose.

An individual is considered as vaccinated against influenza if:

- he or she reports having received an influenza vaccination during the current season;
or
- he or she is registered as vaccinated in the GP information system;
or
- he or she is registered as vaccinated in a vaccination registry;
or
- his or her insurance company can show evidence of pharmacy delivery or re-imburement of influenza vaccine/vaccination during the current influenza season.
or
- has influenza vaccination recorded this season in his/her vaccination card/vaccination booklet.
- Pandemic vaccine: if more than one dose is recommended, the number of doses is documented.



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- Each study site to document:
 - the seasonal and pandemic vaccines used;
 - the precise mode of vaccine ascertainment for each study is specified in the study annexes.

3.9. Confounding factors and effect modifiers

3.9.1. Chronic diseases

If GPs recruiting cases and controls use electronic medical records, the list of ICD codes or classification of Health Problems in Primary Care (ICHPPC-2) codes can be used to document a study participant’s chronic diseases (see Table 1):

Table 1: ICD and ICHPPC-2 codes for chronic diseases

Chronic diseases	ICD code	ICHPPC-2 code
Enlarged spleen, anaemia	280–289, 759.0	B82
Cirrhosis	571	D97
Diabetes and endocrine disease	250, 251	T89, T90
Heart disease	093, 112.81, 130.3, 391, 393–398, 402, 404, 410–429, 745, 746, 747.1, 747.49, 759.82, 785.2, 785.3	K71, K74-77, K81-K84, K86-K87, K99
Hematologic cancer	200–208	B72, B74
Immunodeficiency and organ transplant	042, 079, 279, V08, V42	B99
Lung disease	011, 460, 462, 465, 466, 480–511, 512.8, 513–517, 518.3, 518.8, 519.9, 714.81	A70, R83, R79, R95, R96, R99
Nonhematologic cancer	140–198, 199.1	A79, D74-D78, F74, H75, K72, L71, N74, N76, R84, R85, S77, S79, T71, T73, U75-U77, U79, W72-W73, X75-X77, X81, Y77-Y
Nutritional deficiencies	254, 255, 259.2, 260–269	T05, T99
Renal disease	274.1, 408, 580–591, 593.71–593.73, 593.9	U99
Dementia, stroke	290–294, 331, 340, 341, 348, 438	P70, K90
Rheumatologic diseases	446, 710, 714.0–714.4, 714.8, 714.89, 714.9	L88



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- The exact codes used in each study are specified in the study annexes.

Each patient is evaluated for the presence (currently) of any of the diseases/codes and is classified as 'high risk' if any of them are present.

If ICD or ICHPPC codes are not available, a list of underlying conditions is prepared by using a short questionnaire.

Seasonal vaccine:

The list of underlying conditions in the questionnaire should include at least:

- diabetes, if treated for insulin-dependent or non-insulin-dependent diabetes;
- cardiovascular disease: myocardial infarction, angioplasty, coronary artery bypass surgery, stroke, transient ischemic attacks, treated hypercholesterolemia, treated hypertension;
- chronic pulmonary disease;
- immunodeficiency.

Pandemic vaccine:

The list of underlying conditions in the questionnaire should include all those defining the risk groups in each of the study countries.

- Each study site to specify the list of chronic conditions documented.

3.9.2. Severity

The severity of the underlying conditions is measured by the number of hospital admissions due to the underlying conditions in the 12 months prior to inclusion in the study.

3.9.3. Smoking history

Smoking history is collected and coded as follows: never smoked, former smoker (stopped smoking at least one year before inclusion in the study), current smoker.

3.9.4. Previous influenza vaccinations

Vaccination against seasonal influenza in the last season (recording vaccination information for the previous influenza season). If information on influenza vaccination in other past seasons is available, this can be documented.

3.9.5. Pneumococcal vaccination

Where possible, information on pneumococcal vaccination will be collected, including type of pneumococcal vaccine (e.g. PPSV23 or PCV13) and date or year of receipt.

- Each study site to specify pneumococcal vaccine recommended



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3.9.6. *Functional status*

Low functional status is defined as needing help to bathe or to walk.

- Each study site to specify how they define low functional status.

3.9.7. *Number of GP consultations in the previous 12 months*

In order to document and control for access to care in the various control groups, the number of GP visits in the past 12 months before inclusion in the study is recorded. The consultation for seasonal influenza vaccination should not be included in the count.

3.9.8. *Antiviral administration*

Use of antivirals is documented: type, dosage (if possible) and date of administration (patients receiving antivirals prior to swabbing will be excluded from analysis).

3.9.9. *Source of information*

Data is collected using a standardised questionnaire. For cases and controls selected at GP practices, data are collected face-to-face.

If GPs use electronic medical records, information on collected variables can be extracted from these records to validate the information collected through the standardised questionnaire.

3.10. *Sample size*

Providing VE estimates for each separate study is one of the objectives of this project. Therefore, the minimum sample size should be estimated for each study in order to obtain precise VE estimates. The pooled analyses should not prevent study teams to include a big enough sample size to obtain exact estimates for each separate study.

- The sample size calculation for each study is detailed in the study annexes.

Table 2 illustrates the various sample sizes that would ensure an alpha error of 0.05, a power of 0.8 or 0.9, a detectable odds ratio ranging from 0.3 to 0.9 (OR for A(H3N2) may be low^{13,14,15}), and a vaccine coverage among the source population (or among controls) ranging from 30 to 70 %.

¹³ Skowronski DM et al. Interim estimates of 2014/15 vaccine effectiveness against A(H3N2) from Canada's sentinel physician surveillance network, January 2015. *Euro Surveill.*, 2015 Jan 29;20(4).

¹⁴ Flannery B et al. Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. *MMWR Morb Mortal Wkly Rep.* 2015 Jan 16;64(1):10-5.

¹⁵ Pebody RG. Low effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 mid-season results. *Euro Surveill.* 2015 Feb 5;20(5):21025.



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Table 2: Sample size calculations

Alpha	Power	Controls/ case	Detectable OR	Vaccine coverage in source population/controls	Number of cases	Number of controls
0.05	0.9	1	0.9	0.3	9307	9307
0.05	0.8	1	0.9	0.3	6976	6976
0.05	0.9	1	0.8	0.3	2154	2154
0.05	0.8	1	0.8	0.3	1621	1621
0.05	0.9	1	0.7	0.3	882	882
0.05	0.8	1	0.7	0.3	666	666
0.05	0.9	1	0.6	0.3	454	454
0.05	0.8	1	0.6	0.3	345	345
0.05	0.9	1	0.5	0.3	264	264
0.05	0.8	1	0.5	0.3	202	202
0.05	0.9	1	0.4	0.3	165	165
0.05	0.8	1	0.4	0.3	127	127
0.05	0.9	1	0.3	0.3	109	109
0.05	0.8	1	0.3	0.3	84	84
0.05	0.9	1	0.9	0.4	8059	8059
0.05	0.8	1	0.9	0.4	6040	6040
0.05	0.9	1	0.8	0.4	1844	1844
0.05	0.8	1	0.8	0.4	1387	1387
0.05	0.9	1	0.7	0.4	745	745
0.05	0.8	1	0.7	0.4	563	563
0.05	0.9	1	0.6	0.4	378	378
0.05	0.8	1	0.6	0.4	287	287
0.05	0.9	1	0.5	0.4	216	216
0.05	0.8	1	0.5	0.4	165	165
0.05	0.9	1	0.4	0.4	133	133
0.05	0.8	1	0.4	0.4	102	102
0.05	0.9	1	0.3	0.4	85	85
0.05	0.8	1	0.3	0.4	66	66
0.05	0.9	1	0.9	0.5	7655	7655
0.05	0.8	1	0.9	0.5	5738	5738



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Alpha	Power	Controls/ case	Detectable OR	Vaccine coverage in source population/controls	Number of cases	Number of controls
0.05	0.9	1	0.8	0.5	1731	1731
0.05	0.8	1	0.8	0.5	1303	1303
0.05	0.9	1	0.7	0.5	690	690
0.05	0.8	1	0.7	0.5	522	522
0.05	0.9	1	0.6	0.5	345	345
0.05	0.8	1	0.6	0.5	262	262
0.05	0.9	1	0.5	0.5	194	194
0.05	0.8	1	0.5	0.5	148	148
0.05	0.9	1	0.4	0.5	117	117
0.05	0.8	1	0.4	0.5	90	90
0.05	0.9	1	0.3	0.5	73	73
0.05	0.8	1	0.3	0.5	57	57
0.05	0.9	1	0.9	0.6	7891	7891
0.05	0.8	1	0.9	0.6	5914	5914
0.05	0.9	1	0.8	0.6	1763	1763
0.05	0.8	1	0.8	0.6	1327	1327
0.05	0.9	1	0.7	0.6	694	694
0.05	0.8	1	0.7	0.6	524	524
0.05	0.9	1	0.6	0.6	342	342
0.05	0.8	1	0.6	0.6	260	260
0.05	0.9	1	0.5	0.6	189	189
0.05	0.8	1	0.5	0.6	144	144
0.05	0.9	1	0.4	0.6	111	111
0.05	0.8	1	0.4	0.6	85	85
0.05	0.9	1	0.3	0.6	67	67
0.05	0.8	1	0.3	0.6	52	52
0.05	0.9	1	0.9	0.7	8923	8923

The sample size should be respected for each population subgroup for which a sub (stratified) analysis (e.g. effect modification) is planned.



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3.11. Data

Data on cases and GP controls are collected at GP office level. GPs interview the patients using a standardised questionnaire. GPs using electronic medical records can extract some or all of the variables from these records (e.g. vaccination status, chronic diseases based on ICD codes).

EpiConcept can develop an electronic questionnaire and a web-based questionnaire for participating GPs. Laboratory information will be reported to the study site coordinator using the reporting procedures existing in each study site for influenza surveillance.

Double data entry is recommended unless medical electronic records are used.

Information on antigenic and genetic analyses can be stored separately on an Excel spreadsheet (see Annex 2).

Details on data collection methods, data entry and data transmission are available in the study annexes.

3.11.1. Collected information

Collected information includes (see also Annex 1: List of variables, definition and coding):

- study identification: country and GP;
- case/control demographics;
- ILI signs, symptoms;
- date of onset of ILI;
- date of swabbing;
- laboratory results (including information antigenic and genetic analysis, where available);
- selected underlying chronic conditions (including diabetes, heart disease, chronic obstructing pulmonary disorder and immunodeficiencies);
- number of hospitalisations for the chronic diseases in the previous 12 months;
- number of GP visits in the previous 12 months;
- smoking history;
- current season influenza vaccination including date and product;
- pandemic vaccination including number of doses, date, product (if applicable);
- influenza vaccination in the previous season (or more seasons if available);
- pneumococcal vaccination status, type of vaccine and either date or year of vaccination
- obesity status;
- functional status;
- antiviral administration.

Pandemic vaccine data collected will be revised as more information on the vaccine and the target groups become available.



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3.11.2. Data validation

A sample of paper questionnaires will be checked against the study database to validate data entry.

For GPs using electronic medical records, a sample of questionnaires are checked against the medical records and against the study database.

The agreement between patient vaccine records/vaccination status reported by study participant/vaccine registries is measured.

- The specific validation procedures, including sample size calculation for questionnaire validation (if applicable) are specified in the study annexes.

3.12. Analysis

Analyses are carried out first for each individual study. In a second step, a pooled analysis is conducted (see 3.16 and annex 4).

If sample size permits, analyses are conducted for:

- on all data and separately with cases/control restricted to an interval between date of onset of symptoms and swab taken of <4 days;
- VE against type/subtype-specific influenza, influenza B by lineage and VE by strain-specific genetic groups
- for the various types of vaccines (adjuvanted/non-adjuvanted; trivalent or quadrivalent), groups of vaccines (split virion, subunit, etc.), mode of injection (intradermal vs. intramuscular) and by vaccine product.

All analyses are done separately for seasonal and pandemic vaccine (if applicable).

3.12.1. Individual study site analysis

Descriptive and univariable analysis

The proportion of eligible ILI cases and controls who accepted to participate in the study is calculated (response rate).

Study participants are described by baseline characteristics. Baseline characteristics of cases and controls in unmatched studies are compared using the chi-square test, Fisher's exact test, t-test or the Mann-Whitney test (depending on the nature of the variable and the sample size). The association between vaccination status and baseline characteristics is measured for both case and control groups.

Measure of effect

Vaccine effectiveness is computed as $VE = (1 - OR) * 100$. A 95 % confidence interval is computed around the point estimate.



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Stratified analysis

Analysis is stratified according to (if sample size allows):

- age groups < 75 years and ≥ 75 years;
- presence of at least one chronic condition;
- presence of at least two chronic conditions;
- time: early influenza season, peak, late influenza season.

A sufficient sample size should be planned in order to ensure enough individuals in each stratum for a precise estimate. Effect modification is assessed comparing the OR across the strata of the baseline characteristics. Confounding is assessed by comparing crude and adjusted OR for each baseline characteristic.

Multivariable analysis

A multivariable logistic regression analysis is conducted to control for negative and positive confounding. Odds ratios and standard errors are obtained. Variables are tested for multicollinearity. Interactions are tested using the likelihood ratio test or Wald's test and included in the model if deemed to be biologically plausible and at a reasonable statistical significance (e.g. 5%). If possible, a variable for age and for onset time should always be included in the model.

Continuous variables

Continuous variables in the I-MOVE datasets include age, time of onset of symptoms and GP visits. These variables can be coded as categories, e.g. age group, week of symptom onset, etc. However when coding continuous variables as categories, you may lose information, introduce residual confounding and increase the standard error of your model. Tests will be carried out to see if these variables could be coded as a linear term, polynomial or a spline. In addition, a balance will be sought between simplicity of a model (so a non-expert can understand what is going on), precision and a model that estimates the vaccine effect with the least bias.

If using restricted cubic splines to model continuous variables, the user-written Stata programme "mkspline2" will be used.

Output tables presenting VE estimates

In order to present the results in the most transparent manner and to enable the reader to best understand the data, tables similar to the table below can be used. Useful information includes numbers of cases and controls (overall and vaccinated) and presentation of results for different models.



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Type/subtype	Analysis scenarios, population included		VE (%)	(95%CI)
A(H1N1)pdm09		N (cases/ vaccinated; controls/ vaccinated)		
		Crude *		
		Adjusted for sex*		
		Adjusted for chronic condition*		
		Adjusted for age (cubic spline)*		
		Adjusted for onset week, age (cubic spline)*		
		Adjusted for onset week, chronic condition*		
		Adjusted for onset week, age (cubic spline), chronic conditions, sex *		
	<75 years			
		N (cases/ vaccinated; controls/ vaccinated)		
		Crude*		
		Adjusted for onset month, age (cubic spline), chronic condition		
	>74 years			
		N (cases/ vaccinated; controls/ vaccinated)		
		Crude*		
		Adjusted for onset week, age (cubic spline), chronic condition, sex *		

* If pooled analysis, study site included as fixed effect.



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Further analyses

Where sample size allows, further analyses will be carried out. These include:

- VE at different time points along the season (e.g. VE by week or group of weeks in the season [VE for weeks 2-3, 4-5, 6-7, etc.])
- VE by time since vaccination (Time since vaccination can be calculated by subtracting the date of vaccination from the date of onset. Time since vaccination can then be modelled as a continuous variable (see Annex 8 for further information on VE by time since vaccination)
- VE of previous season influenza vaccination only, current season influenza only and combined season vaccination.
- Using the systematic samples of the sequenced isolates, the proportion of virus changes will be calculated at different time points along the season (e.g. by week or group of weeks in the season: weeks 2-3, 4-5, 5-6, etc.). This will be compared to VE at different time points along the season.
- As a sensitivity analysis, VE will be calculated excluding those vaccinated <15 days before onset of symptoms (in the main analysis those records are coded as “unvaccinated”)

Minimal sample size

Sample sizes may be very small for some subanalyses. Different criteria can be used to determine if sample size is high enough to obtain a valid measure of vaccine effectiveness:

- There are at least 10-15 cases (or controls, whichever is smaller) in the subanalysis
- There are ≥ 5 records in each cell of the two by two table of case and vaccination status
- The precision of the estimate does not span both -200% and 90%.

Each study site to specify criteria for which to determine minimum sample size if desired.

Missing data

Any missing data will be documented.

If many data are missing and/or there is evidence of bias in the missing data, and variables that are considered good predictors of the missing data are available, then multiple imputation methods at study level will be used to replace missing values.

A sensitivity analysis will be carried out comparing results from complete case analysis (where records with missing data are dropped) and full set analysis (with imputed data).



European Union

3.13. Data management

3.13.1. Individual analysis

EpiConcept provides the option of web-based data collection methods, if so desired by the countries. These methods can also be combined with paper-based methods.

If the EpiConcept web-based data collection methods are not used, data can be coded as outlined in Annex 1, but it is not required.

3.13.2. Data cleaning

Summary and frequency tables as well as visual representations of appropriate variables are used to find illegal, implausible or missing values within the dataset. Checks for inconsistencies are carried out (e.g. date of swabbing before date of onset of symptoms). These values are checked against the questionnaires or queried with the GP. Any changes to the data are documented and stored separately from the crude database. Any recoding of data (e.g. age) is documented. A guide and/or an example Stata do-file for data cleaning is provided if so desired.

3.13.3. Pooled analysis

EpiConcept conducts the pooled analysis. Individual data from each study is sent to EpiConcept's study database. All personal identifier information such as names, addresses, and medical registration codes are deleted before data transmission to EpiConcept, where all individual data are pooled. A country (or study) identifier is included in each record (e.g. ES for Spain, UK for the United Kingdom), a GP code is included (e.g. a unique number), and each record is given a unique number. This number is also included in the study team's database and will be used by EpiConcept and the study teams during pooling, so that records can be traced back whilst maintaining anonymity, if there are any further queries. Study databases can be sent to EpiConcept in any format. Data can be coded as outlined in Annex 1, or a codebook can be provided by the study teams to EpiConcept that includes the variable names, descriptions and coding. EpiConcept performs all necessary data cleaning. EpiConcept documents and shares any further data cleaning and analysis with all study coordinators to ensure it can be reproduced.

See annex 4 for detailed guidelines to the pooled analysis.

3.14. Potential biases

3.14.1. Negative confounding

Negative confounding refers to biases that reduce the VE estimate if not accounted for. For example high risk groups are more likely to be vaccinated may be more likely to get flu (or more severe symptoms).



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3.14.2. Positive confounding

Positive confounding refers to biases that increase the VE estimate if not accounted for. For example a 'healthy vaccine effect'. People with a healthy lifestyle may be more likely to accept/request vaccination and less likely to get flu (although they might actually be more likely to consult with ILI).

Positive and negative confounding is minimised through stratification and multivariable analysis and variables collected in order to measure positive and negative confounding. It is also reduced by the use of the study design properties of the TND as this takes into account factors associated with the propensity to consult. It is not possible to rule out the presence of characteristics in the study population for which no information is collected in the study questionnaire and that therefore could lead to positive or negative confounding. In this way, residual positive or negative confounding may be present.

3.14.3. Representativity of cases

The study includes only cases consulting a GP for ILI. Health seeking behaviour may differ by country depending on the case management strategy (e.g. recommendation of not going to the GP). In some countries, only severe cases will go to the GP. In other, severe cases will directly go to the emergency rooms without consulting their GP. The type of cases included in the study should be described for each of the studies and how its representativeness may affect the VE estimates.

3.14.4. Pooled estimate and its bias

Any bias in the individual studies influences the pooled estimate. The power of the test for the presence of heterogeneity between individual studies is low if there are few studies. In this case, the test may not be able to detect heterogeneity between studies although heterogeneity is present. It is important that heterogeneity is assessed using qualitative knowledge about differences between studies. Depending on the nature of the bias, the inclusion of biased studies in the pooled estimate could lead to over- or underestimation of the true vaccine effectiveness.

3.15. Dissemination of results

The enrolment of cases/controls is regularly updated by each study coordinator on the 'I-MOVE+' web page.

Seasonal vaccine: first VE estimates (intra-seasonal) are disseminated early during the influenza season; final estimates follow at the end of the season.

Pandemic vaccine: first estimates will be disseminated once the sample size would allow for meaningful interpretation.

3.15.1. Publications, scientific communication

Each study coordinator decides where the results of the individual studies are published and which scientific conferences are attended in order to present the results. An article presenting the results of the pooled analysis and estimates for the EU/EEA is submitted to a peer-reviewed journal. Regarding



European Union

the list of authors, we will follow the recommendations of the International Committee of Medical Journal Editors <http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html> and each season the list of authors will be defined in the Principles of Collaboration. Co-workers and contributors are acknowledged.

I-MOVE+ results will contribute to the report prepared by the GIVE (Global Influenza Vaccine Effectiveness) collaboration for the annual Northern and Southern Hemisphere WHO Meeting on the Composition of Influenza Virus Vaccines.

3.16. Training

Participating GPs are trained on the study protocol before the start of the study. They receive the protocol, questionnaires and laboratory swabbing procedures.

4. Logistical aspects

4.1. Study leader

In each country, a principal investigator coordinates the study at the country level and acts as focal point for the European study. EpiConcept is in charge of the pooled analysis.

4.2. Human resources

In each country, a part-time investigator is in charge of monitoring data collection at the GP office level. GPs collect information among cases and controls. GPs could be offered a payment or compensation for their participation in the study.

- The specific human resources needed in each country are detailed in the study annexes. EpiConcept *ensures the overall coordination of the various studies.*

4.3. Supervision

Site visits and joint workshops may be organised by EpiConcept/Member States consortium in order to carry out an appraisal of the ongoing studies in the various countries involved. The appraisal team is composed of two persons from the various project partners.

4.4. Questionnaires

Standardised questionnaires are developed for the study. The variables used at the European level are collected in the same way for each of the studies (see Annex 1: List of variables, definition and coding).



European Union



4.5. Computer support

Data collection and entry is conducted at the country level. For countries willing to submit data electronically, EpiConcept provides an online questionnaire.

4.6. Consent

Each study complies with national ethics committee requirements. Informed consent is required from all participants. The national ethics committees specify whether oral or written consent is required.

- Details are available in the study annexes.

4.7. Report

Each study site will write a report at the end of the season and submit it to the study coordination team. EpiConcept will write a final report presenting the results of the pooled analysis.



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European Union

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Annexes

Annex 1: List of variables, definitions and coding

Variable name	Type	Values and coding	Definition
idcountry	Numeric	Coded according to international country codes	Identifier uniquely identifying the country
participate	Numeric (binary)	0 = No 1 = Yes	Agrees to participate
refuse	Text		Reasons for refusal to participate
id	Numeric (continuous)	Unique integer	Unique number for each record
case	Numeric (binary)	0 = control 1 = case	Identifies cases and controls
gpcode	Numeric (continuous)	Unique integer	Unique number for each GP (preventing identification of GP)
dob	Date	dd/mm/yyyy	Date of birth of study participant
age	Numeric (continuous)	Integer	Age of each participant in years
sex	Numeric (binary)	0 = female 1 = male	Sex of study participant
onsetdate	Date	dd/mm/yyyy	Date of onset of symptoms
swabdate	Date	dd/mm/yyyy	Swabbing date
fever	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Fever
malaise	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Malaise
myalgia	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Myalgia
cough	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cough
sorethroat	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sore throat
suddenonset	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sudden onset
headache	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Headache
shortness of breath	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Weakness



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Variable name	Type	Values and coding	Definition
lab_res	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result (positive/negative)
lab_virusa	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus type A
lab_virusb	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus type B
lab_h1n1	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus subtype AH1N1
lab_h3n2	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus subtype AH3N2
lineage	Numeric (categorical)	0 = Yamagata 1 = Victoria 8 = Do not know	Laboratory result: B virus lineage
genetic_group	Text		Laboratory result: genetic group
antigenic_analysis	Text		Laboratory result: antigenic group
seasvaccany	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination in current season
seasvacdate	Date	dd/mm/yyyy	Vaccination date
seasvacctype	Text		Type of vaccine (product name)
pneumovacc	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received pneumococcal vaccination
pneumovacctype	Numeric (categorical)	1 = PPSV23 2 = PCV13 3 = Other (pls specify) 8 = Do not know	Type of pneumococcal vaccine
pneumovacctype_other	Text		Other type of pneumococcal vaccine if not PPSV23 or PCV13
pneumoyear	Number		Year of receipt of pneumococcal vaccination
vacc_14	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Previous influenza vaccination 2014-15
diabetes	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Diabetes and endocrine
heart_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Heart disease
immuno	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Immunodeficiency and organ transplant



European Union

Variable name	Type	Values and coding	Definition
lungdis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Lung disease
obese	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Clinically obese
severity	Numeric (count)	integer	Number of hospitalisations previous 12 months for the chronic disease
gpvisit	Numeric (count)	integer	Number of GP consultations previous 12 months
fs_bath	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Requires assistance to bath
fs_walk	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Requires assistance to walk
smoking		0 = Never 1 = Former 2 = Current 9 = Do not know	Never, former (stopped smoking at least 1 year before inclusion in the study), current smoker
antivir	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Administration of antivirals
antivirdate	Date	dd/mm/yyyy	Date administration antiviral
antivirtype	Text		Type of antiviral (brand name)
res_home	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Exclusion criteria: living in a residential home
contra	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Exclusion criteria: contraindication for influenza vaccination
prev_flu	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Lab-confirmed previous influenza in the season

This table represents a selection of confounders. Variables can be included or excluded as necessary.

In a pandemic, these further variables may be required:

Variable name	Type	Values and coding	Definition
panvaccany	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination 2009-10
panvaccd1	Date	dd/mm/yyyy	Vaccination date first dose
panvaccd2	Date	dd/mm/yyyy	Vaccination date second dose
panvacctype	Text		Type of vaccine (product name)



European Union

Variable name	Type	Values and coding	Definition
panvaccdose	Numeric	0, 1, 2	Number of doses received

The variables above can be recoded to obtain variables useful for analysis. Possible ways to recode variables are outlined in the pooled data analysis section in annex 5.

Annex 2: Genetic and antigenic analysis data (examples)

A(H1N1)	Country	Region /City	ID number I-MOVE+ case-control study	Date sample	Strain							GISAID number	Antigenic analysis (IHA)	Genetic analysis (HA1)	Genetic group
						83	97	163	185	203	...				
Row for 2014/15 vaccine reference strain															
Row for strain with AA substitutions compared with vaccine reference strain															
Row for strain with AA substitutions compared with vaccine reference strain															

A(H3N2)	Country	Region /City	ID number I-MOVE case-control study	Date sample	Strain							GISAID number	Antigenic analysis (IHA)	Genetic analysis (HA1)	Genetic group	
						122	128	142	145	157	198	225				
Row for 2014/15 vaccine reference strain																
Row for strain with AA substitutions compared with vaccine reference strain																



European Union

B(Yamagata)	Country	Region/City	ID number I-MOVE case-control study	Date sample	Strain											GISAIID number	Antigenic analysis (IHA)	Genetic analysis (HA1)	Genetic group
						48	50	108	116	150	165	202	229	298	312				
Row for 2014/15 vaccine reference strain																			
Row for strain with AA substitutions compared with vaccine reference strain																			

B(Victoria)	Country	Region/City	ID number I-MOVE case-control study	Date sample	Strain	Genetic analysis (HA1)
Row for 2014/15 vaccine reference strain						
Row for strain with AA substitutions compared with vaccine reference strain						

Annex 3: Pooled data management

Data preparation and transfer at study-level

Data validation, cleaning and verification will be carried out at study-level (for flow chart, see Annex 5). All personal identifier information such as names, addresses, medical registration codes will be deleted. A country (or study) identifier (e.g. ES for Spain, RO for Romania) and a unique code to identify a practitioner will be included in each record and each record will be given a unique number. This unique identifier is also included in the study team database and will be used by EpiConcept and the study teams during pooling, so that records can be traced back whilst maintaining anonymity, if there are any further queries.

Minimum dataset

The minimum dataset will be transmitted to EpiConcept where individual data will be pooled, and includes:

- study identification: country and GP
- case / control demographics
- signs, symptoms
- date of onset of ILI
- date of swabbing
- laboratory results
- selected underlying chronic conditions relating to country specific influenza vaccine recommendations
- number of hospitalisations for the chronic diseases in the previous 12 months;
- obesity status;
- current season influenza vaccination status including date received and product; and
- previous season influenza vaccination status.

Further recommended variables

- Number of GP visits in the previous 12 months
- Smoking history
- Pneumococcal vaccine status, pneumococcal vaccine type and year of vaccination
- Antiviral administration (including dates of administration)

Data transfer

Study databases can be sent to EpiConcept in any format (e.g. Stata, CSV, EpiData, etc.). The minimum dataset can be coded as in Annex 1, or a codebook can be provided to EpiConcept with the variable names and descriptions and the coding of variable values.

Data cleaning

EpiConcept will carry out data cleaning again, once the data are received. Summary and frequency tables, logical error checks and graphic displays of appropriate variables will be used to find illegal, implausible or missing values within the dataset. Checks for inconsistencies will be carried out (e.g. date of swabbing before date of onset of symptoms). Any improbable, illegal or missing values will be reported to the country in question.



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Any subsequent changes to the data will be fully documented and stored separately from the crude database, to ensure reproducibility and transparency of data management.

Data exclusion and restriction

- Patients that fulfil the exclusion criteria (contra-indications for vaccination, institutionalised patients, unable to give informed consent) will be excluded from the analysis.
- The data will be further restricted, with patients included only if the following criteria apply:
- no antivirals administered prior to swabbing
- day of onset of symptoms more than 14 days after begin of national vaccination campaign
- symptoms correspond to the EU ILI case definition
- patients swabbed within 7 days of symptom onset
- week of onset of symptoms not before the week of onset of the first influenza positive case in the study
- week of onset of symptoms not after the week of onset of the last influenza positive case in the study, or the week of onset of an influenza positive case after which there were 2 consecutive weeks of no cases
- lab results are available

A study-site specific flowchart of exclusions and restrictions will be shared with each of the study sites.

Data recoding

Variables will be recoded and new variables generated according to Annex 6. The recoded data will be stored separately from the crude data and recoding will be documented.

Missing data

In the first instance (at country level), great care will be taken to avoid missing data.

Missing data will be described in terms of number and frequency of missing values for each variable of interest and in terms of number and frequency of records with 0, 1, 2, etc. missing values.

Baseline characteristics of records with missing data will be compared to records without missing data.

Each variable with missing data will be qualitatively assessed to determine the mechanism of missing data.

Reasons for missing data for each variable will be discussed with all study partners. The mechanism for missing data will be determined: missing completely at random, missing at random and not missing at random. If data are determined to be missing at random, then an imputation could be carried out.



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If there is a reasonable amount of missing data, associations of the missing data with the other covariates in the study will be described. If there is at least one variable with missing data associated with the outcome and with an exposure/risk factor then a complete case analysis might be biased.

If this is the case and if variables that are considered good predictors of the missing data are available, then multiple imputation methods at study level will be used to replace missing values.

If there are very few missing data, an imputation will not be carried out.

A complete case analysis will be carried out and presented even if multiple imputation methods are applied on the dataset.

Multiple imputation

Multiple imputation is a procedure where missing data are estimated from the observed and the uncertainty of the missing data is taken into account in the final estimate.

Here, multiple imputation using chained equations will be carried out, using the Stata “mi impute chained” procedure. The imputation is done by creating a number of possible databases and using a pooled analysis (taking into account within- and between-database variance) to obtain final estimates. This takes the uncertainty of the imputation of missing data into account. Variables to include in the imputation model will include:

- Both variables with and without missing data
- The outcome variable
- If there is a measure of time in the dataset, it will be included
- All variables that are part of the multivariable model
- Other variables that are predictive of missing data and of a value to be missing
- Variables predictive of missing values

Different models for imputation, e.g. including different sets of predictors, different numbers of iterations and databases created will be compared and the robustness of the imputation assessed.

After imputation, the imputed data will be assessed in the following way

- Checked for any incomplete data in the imputed variables
- Imputed percentages for each variable compared to observed percentages (to check for very large differences, implausible values, etc.)

After carrying out a multiple imputation, the resulting dataset will be analysed with specific multiple imputation commands.

The results from the complete case analysis will be compared to the analysis using the imputed dataset.



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Data appending and data flow

After data cleaning the data will be appended, and a unique identifier for each GP per country will be created by concatenating the study code and the GP code. An example data flow chart is presented in Annex 5.



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Annex 4: Pooled data analysis

Descriptive analysis

The main characteristics of each study will be summarised individually, including:

- Number of GPs participating and catchment population
- Beginning of the study
- Beginning of influenza period, peak, end
- Beginning of vaccination campaigns for seasonal and pandemic vaccine (if applicable)
- Proportion of ILI flu positive among all ILI cases
- Proportion of persons belonging to target group for vaccination
- Sample size, including cases and controls by vaccination status

Individual level analysis

Analyses will be carried out first for each individual study, shared with the study site team for validation, and then, in a second step, a pooled analysis will be conducted.

Analysis will be done if sample size permits

- on all data and separately with cases/control restricted to an interval between date of onset of symptoms and swab taken of <4 days;
- for VE against type/subtype-specific influenza, influenza B by lineage and VE by strain-specific genetic groups
- for the various types of vaccines (adjuvanted/non-adjuvanted; trivalent vs. quadrivalent), groups of vaccines (split virion, subunit, etc.), mode of injection (intradermal vs. intramuscular) and by vaccine product.
- By age group (<75 years or >74 years)

All analyses will be carried out with Stata v13 (Stata Corp LP, College Station, TX, USA).

For methods on individual level analysis, see main section.

Testing for heterogeneity

Study-specific crude and adjusted ORs and their CIs will be plotted in separate forest plots. Following the core protocol minimises heterogeneity between studies. However adherence to the protocol and study design and study quality characteristics will be checked again. Other study site characteristics will be assessed where feasible, such as types of circulating virus, information on health care use, organisation of the vaccination campaign. Then a qualitative decision will be taken if one or more studies are substantially different from the other. Statistical heterogeneity between studies will be tested using Q-test and the I² index (see boxes for formulae below). The Q statistic follows a Chi² distribution (with k-1 degrees of freedom). The Q-test reports presence or absence of heterogeneity, while the I² index (based on the Q-statistic) quantifies the extent of the heterogeneity.



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According to the Higgins and Thompson classification, an I² index of around 25% indicates low, 50% indicates medium and 75% indicated high heterogeneity between studies.

$$Q = \sum w_i (\log(OR_i) - \log(OR_F))^2$$

Where:

$$w_i = 1/v_i$$

v_i is the inverse variance of the estimated log odds ratio of study i.

$$\log(OR_F) = \frac{\sum w_i \times \log(OR_i)}{\sum w_i}$$

$$I^2 = \frac{Q - (k - 1)}{Q} \times 100\% \quad \text{for } Q > (k - 1)$$

$$I^2 = 0 \quad \text{for } Q \leq (k - 1)$$

Formulae are given here for completeness, in practice these measures are automatically calculated by many statistical software packages as part of the meta-analysis commands.

1-stage pooled analysis approach

If sample sizes are too small to measure vaccine effectiveness controlling for all potential confounders for each individual study site, a 1-stage pooled approach will be used for analysis.

Individual study data will be pooled into one dataset and analysed as a 1-stage model with study as a fixed effect (see Annex 7 for Stata syntax). This could provide a large enough sample size to obtain (for example) an estimate of VE early in the season with reasonable precision. The results of this analysis should be interpreted with caution, though, as it assumes not only that the underlying true exposure effect is the same in all studies, but also that the association of all covariates with the outcome is the same in all studies. If the latter is not the case, then interactions between study sites and covariates need to be introduced.

Formal tests of interaction between study site and covariates will be carried out to determine if the effect of each covariate differs across studies, to test the assumptions of the 1-stage pooled fixed effect analysis. Of course, the significance of interaction terms are themselves influenced by sample size and should be interpreted also with caution. Particular care needs to be taken if heterogeneity is found between study sites when using a 1-stage fixed effects approach (see above section). Reasons for heterogeneity need to be thoroughly investigated and the assumptions underlying the 1-stage pooling approach need to be revisited.



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Two-stage pooling approach

If adequate sample size by study is achieved to obtain an adjusted OR by study site individually, then a 2-stage approach to pooled analysis will be taken.

Study-specific adjusted ORs and standard errors for the effect of current influenza vaccination obtained from the individual studies, will be combined in a model that incorporates random effects of the studies, to account for unmeasured country- and study-specific factors that differ between studies.

The study-specific exposure-disease effects (ORs) are then weighted by the inverse of their marginal variances. The marginal variance is the sum of the individual study-specific variances and the variance of the random study effects (τ^2). This will give the pooled odds ratio and standard error. See Annex 7 for an example of Stata syntax.

$$\log(OR_R) = \frac{\sum w_i^* \times \log(OR_i)}{\sum w_i^*}$$
$$w_i^* = \frac{1}{v_i + \tau^2}$$

The study specific ORs and their CIs, along with the pooled odds ratio will be presented graphically in a forest plot. This model will also be compared against a 2-stage analysis with fixed study effects, to assess the effects of model assumptions.

If despite the common protocol covariates were not uniformly collected in the different studies, then an analysis will be carried out excluding certain studies and a comparison to the analysis including all studies will be made. In a different scenario, analyses can also be carried out excluding certain study participants for whom variables were collected differently.

Pooled analysis

The following analyses (1-stage or 2-stage depending on sample size) will be carried out if sample size permits:

- on all data and separately with cases/control restricted to an interval between date of onset of symptoms and swab taken of <4 days;
- for VE against type/subtype-specific influenza, influenza B by lineage and VE for strain-specific genetic groups
- for the various types of vaccines (adjuvanted/non-adjuvanted; trivalent vs. quadrivalent), groups of vaccines (split virion, subunit, etc.), mode of injection (intradermal vs. intramuscular) and by vaccine product.

All analyses are done separately for seasonal and pandemic vaccine (if applicable).



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Analyses by vaccine product will include only countries (or regions if the information is available) where the vaccine product is available. Countries (or regions if information is available) where the vaccine product is not available will be excluded from the analysis.

Stratified analysis

Analysis is stratified according to (if sample size allows):

- age groups < 75 years and > 74 years;
- presence of at least one chronic condition;
- presence of at least two chronic conditions;
- time: early influenza season, peak, late influenza season.

Further pooled analyses

Where sample size allows, further analyses will be carried out. These include:

- VE at different time points along the season (e.g. VE by week or group of weeks in the season [VE for weeks 2-3, 4-5, 6-7, etc.]
- VE by time since vaccination (Time since vaccination can be calculated by subtracting the date of vaccination from the date of onset. Time since vaccination can then be modelled as a continuous variable (see Annex 8 for further information on VE by time since vaccination)
- VE of previous season influenza vaccination only, current season influenza only and combined season vaccination. Where possible, influenza vaccination information from seasons more than one year before the current season will be taken into account.
- Using the systematic samples of the sequenced isolates, the proportion of virus changes will be calculated at different time points along the season (e.g. by week or group of weeks in the season: weeks 2-3, 4-5, 5-6, etc.). This will be compared to VE at different time points along the season.
- As a sensitivity analysis, VE will be calculated excluding those vaccinated <15 days before onset of symptoms (in the main analysis those records are coded as “unvaccinated”)

Controlling for GP effect

Primary analysis will be carried out using simple logistic regression to obtain the individual study estimates. However there could be an effect of GP that is related both to the exposure (propensity to vaccinate) and the outcome (in terms of swabbing behaviour). To adjust for this cluster effect, a multi-level logistic regression with each GP as a random effect will be carried out when using a 1-stage pooled analysis.



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Multi-level logistic regression can also be carried out for each individual study with GP as a random effect. Then the 2-stage model as outlined above will be used to obtain a summary VE measure, using these estimates.

The same applies to stratified analyses. The point estimates and confidence intervals from the multi-level and simple logistic regression will be compared in a sensitivity analysis.

Continuous variables

Continuous variables in the I-MOVE datasets include age, time of onset of symptoms and GP visits. These variables can be coded as categories, e.g. age group, week of symptom onset, etc. However when coding continuous variables as categories, you may lose information, introduce residual confounding and increase the standard error of your model. Tests will be carried out to see if these variables could be coded as a linear term, polynomial or a spline. In addition, a balance will be sought between simplicity of a model (so a non-expert can understand what is going on) and a model that is most precise.

If using restricted cubic splines to model continuous variables, the user-written Stata programme “mkspline2” will be used.

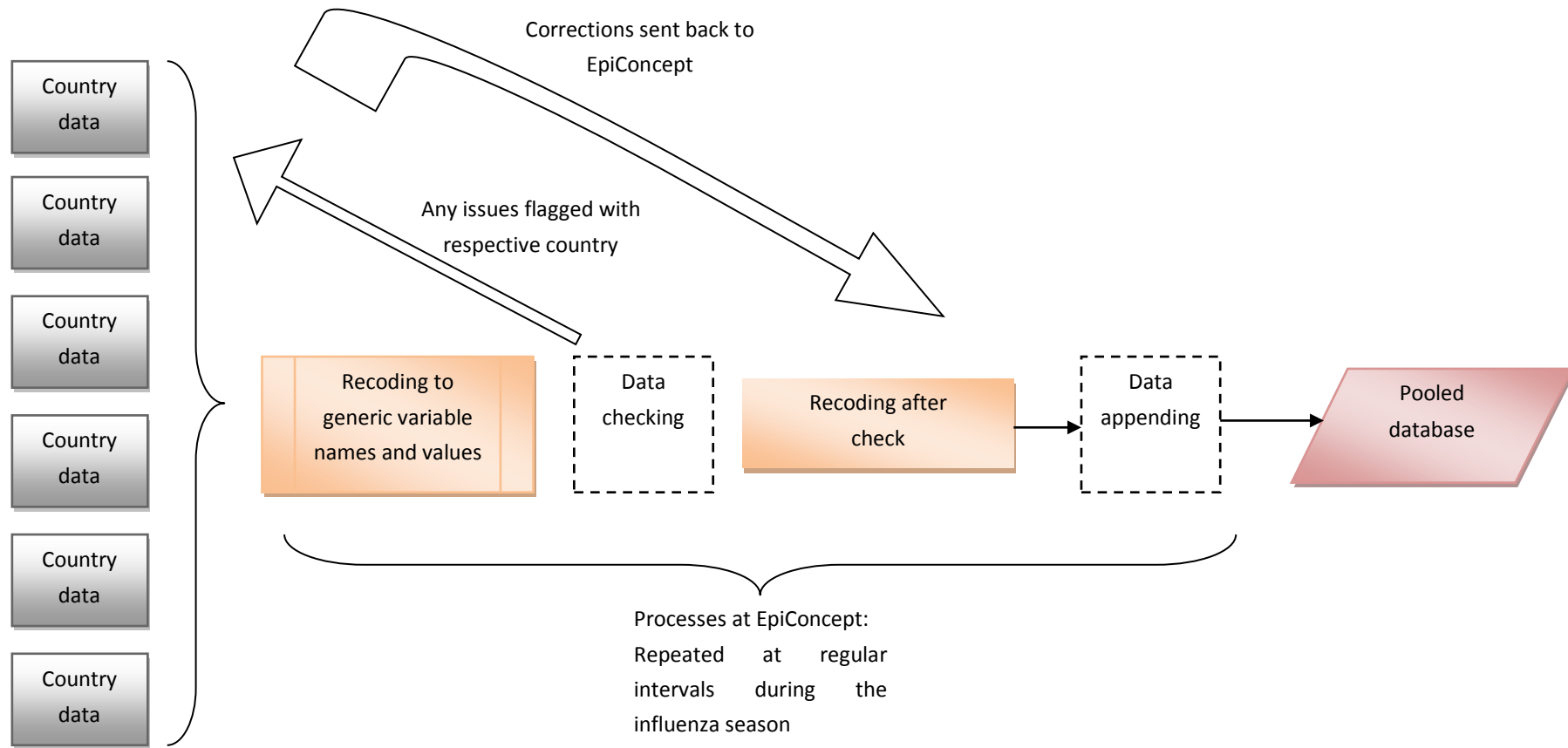
GP level data

If available, study sites will provide data by number of ILI seen by GP by age group and numbers swabbed, in order to assess compliance to the protocol. In addition, if not all elderly meeting the ILI case definition are swabbed by the GPs, then we can calculate a sampling fraction by GP to correct for this.



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Annex 5: Data flow for pooled database



Countries send their individual data to EpiConcept according to minimum dataset guidelines



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Annex 6: Generated/recoded variables

Variable name	Type	Values and coding	Definition
cases	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for any influenza type.
casea	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for any influenza A type.
caseh1	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for influenza type A(H1N1).
caseh3	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for influenza type A(H3N2).
caseb	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for any influenza B type (regardless of lineage).
caseby	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed influenza B Yamagata lineage.
casebv	Numeric (binary)	0 = No 1 = Yes	Indicates ILI case that is lab-confirmed for influenza B Victoria lineage.
ili	Numeric (binary)	0 = No 1 = Yes	Variable that corresponds to EU ILI case definition (coded using the symptoms in dataset)
svaccdelay	Numeric (continuous)	Integer	Number of days between seasonal flu vaccination date and onset date of symptoms (needs to be modified if 2 doses are required)
svacc	Numeric (binary)	0 = No 1 = Yes	Coded as yes if >14 days between seasonal vaccination and onset of symptoms
swabdelay	Numeric (continuous)	Unique integer	Number of days between onset date of symptoms and swab date
swabless4	Numeric (binary)	0 = No 1 = Yes	1 indicates less than 4 days between symptom onset and swab date. 0 indicates more than 3 days.



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Variable name	Type	Values and coding	Definition
anychron	Numeric (binary)	0 = No 1 = Yes	0 indicates no chronic disease for which flu vaccination is recommended 1 indicates at least 1 chronic disease for which flu vaccination is recommended
numchron	Numeric (continuous)	Unique integer	Number of chronic diseases reported for the patient.
twochron	Numeric (binary)	0 = No 1 = Yes	0 indicates no or only one chronic disease for which flu vaccination is recommended 1 indicates at least 2 chronic diseases for which flu vaccination is recommended
smokcurr	Numeric (binary)	0 = No 1 = Yes	Current smoker (1) vs. former or never smoker (0).
hosp_bin	Numeric (binary)	0 = No 1 = Yes	Not hospitalized for chronic disease in past 12 months (0), hospitalized for chronic disease in past 12 months (1)
gpvisitgp	Numeric (categorical)	0 = 0-1 visit 1 = 2-4 visits 2 = 5+ visits	The continuous variable GP visit is grouped into categories.
agegp10	Numeric (categorical)	0 = 65-74 years 1 = 75-84 years 2 = 85+ years	The continuous variable age is grouped into 10 year age groups, (although often splines are used for analysis of this continuous variable)
agegroup	Numeric (categorical)	0 = 65-74 years 1 = 75-max years	The continuous variable age visit is grouped into 2 age groups, used for stratification.
Onsetweek1	Continuous	Integer	Week of onset of ILI symptoms, coded according to ISO weeks
adj	Numeric (categorical)	0 = Not vaccinated 1 = Non-adjuvanted 2 = Adjuvanted 8 = Vaccinated, product unknown, 9 = Vaccination status unknown	Persons with adjuvanted vaccine received >14 days before symptom onset are coded as 1, those who received non-adjuvanted vaccine >14 days before symptom onset are coded as 2 and those unvaccinated or vaccinated <15 days before symptom onset are coded as 0.



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Variable name	Type	Values and coding	Definition
vaccgroup	Numeric (categorical)	0 = Not vaccinated 1 = Inactivated subunit (egg mediated) 2 = Inactivated split virion (egg mediated) 3 = Adjuvanted 4 = Inactivated subunit (cell mediated) 8 = Vaccinated, unknown product 9 = Vaccination status unknown	Classification of the different vaccine groups
vaccval	Numeric (categorical)	0 = Not vaccinated 1 = Vaccinated with trivalent vaccine 2 = Vaccinated with quadrivalent vaccine 8 = Vaccinated, product unknown, 9 = Vaccination status unknown	Persons with trivalent vaccine received >14 days before symptom onset are coded as 1, those who received quadrivalent vaccine >14 days before symptom onset are coded as 2 and those unvaccinated or vaccinated <15 days before symptom onset are coded as 0.
vaccmode	Numeric (categorical)	0 = Not vaccinated 1 = Vaccinated intramuscularly 2 = Vaccinated intradermally 9 = Vaccination status unknown	Mode of vaccination



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Annex 7: Stata syntax

Syntax for 2-stage pooling model:

```
// using pooled dataset with a variable for study
gen study=""
gen logor=.
gen or=.
gen logse=.
```

// With the loop below we are calculating the OR, the log OR and the log standard error for each study. Only these data will be used for the 2-stage pooled analyses.

```
local i=1
foreach country in country1 country2 country3 country4 {           // replace "countryn" with country/study abbreviation
logistic cases svacc i.agegroup sex anychron smokcurr hosp_bin gpvisit i.onsetweek1 if idcountry=="`country'"
matrix b = e(b)
matrix se = e(V)
replace study="`country'" in `i'                                  // here we are creating a summary dataset with 1 row per study
replace logor= b[1,1] in `i'
replace logse=sqrt(se[1,1]) in `i'
replace or=exp(b[1,1]) in `i'
local ++i
}
```

// Dropping data, so only the variables interesting for the 2-level model remain:

```
keep if study!=""           // now our dataset only has 1 line per study
save twostage.dta, replace
metan logor logse, effect(Odds ratio) eform xlabel(0.25, 0.5, 1, 1.25, 1.5) textsize(250) label(namevar=study) randomi
// Above is the meta-analysis command that uses the log OR and log SE to carry out a 2-stage random effects pooled analysis
// Outputs are the individual and pooled OR estimates and confidence intervals as well as a forest plot
```

Syntax for 1-stage pooling model:



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```
// using pooled dataset with a variable for study  
xi: logistic cases svacc i.agegroup sex anychron smokcurr hosp_bin gpvisit i.onsetweek i.idcountry
```

Stata syntax serves as guidance only and syntax should be adapted to the given situation



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Annex 8: Measuring VE by time since vaccination

Time since vaccination as exposure of interest

VE will be measured using vaccinated against influenza at any time point that is more than 14 days before symptoms onset. Additionally, we will look at VE according to time since vaccination.

We will use two methods to code time since vaccination:

- 1) Time since vaccination will be coded as a categorical variable, with unvaccinated (no vaccine received or vaccinated <15 days) as a category, and two other further categories. If sample size allows, these categories will be defined as \leq three months between vaccination and onset and $>$ three months between vaccination and onset. Keeping the same category between seasons will allow greater comparability. If sample size allows, then three further categories could be considered, at three and four months, or two and four months. The determination of these categories needs to be determined with more knowledge of the data. If sample size does not allow the categorisation of \leq three months and $>$ three months, then the median days between vaccination and symptoms may be chosen. If sample size allows, the categories could include not vaccinated, 1-7 days between vaccination and onset of symptoms and 8-14 days between vaccination and onset of symptoms (or 0-7 days and 8-14 days if sample size is small). Note if this categorisation is used, then the study period would begin at time of vaccination campaign, rather than 15 days after campaign, providing the virus is circulating. This categorisation may be most feasible for the year of the pandemic, where vaccination campaigns coincided with circulation of influenza.
- 2) Time since vaccination will be coded as a continuous variable, with time since vaccination coded as date of onset of symptoms minus date of vaccination. We will use a cubic spline, tail-restricted at the upper end to model time since vaccination. Four knots are planned to be used for the spline, with knots at 0 and 15 days and two further knots at the 40th and 90th percentile. All persons not receiving vaccine will be coded as "0". The value of time since vaccination (date of symptom onset minus date of vaccination) will also be included for those vaccinated less than 15 days before symptom onset. They will not be considered "unvaccinated" for this analysis.



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Data Analysis

- To measure influenza vaccine effectiveness by time since vaccination across each season for each circulating influenza type/subtype, and compare differences in VE at different times since vaccination, in order to determine if there is a reduction in influenza VE with different times since vaccination.
- To measure influenza vaccine effectiveness by time since vaccination across each season for each circulating influenza type/subtype, stratified by early/late phase within the influenza season, to determine if there is lower VE by time since vaccination regardless of the time period within the influenza season.
- To carry out the above two analyses by age group, in order to determine if changes in VE differ by age group.

Analysis using Time since vaccination as a categorical variable

Time since vaccination will be modelled into categories.

Crude and multivariable analyses will be carried out with time since vaccination as a categorical variable, for the overall influenza period and by influenza phase.

A table similar to the following will be completed:

Influenza phase	Crude vs adjusted	Delay between vaccination and symptom onset	Cases and controls (N/N)	Vaccine effectiveness (%)	95% Confidence intervals
Overall	Crude ^a	=<3 months			
		>3 months			
	Adjusted model ^{a,b}	=<3 months			
		>3 months			
Adjusted model ^{a,c}	=<3 months				
	>3 months				
Adjusted model ^{a,d}	=<3 months				
	>3 months				
First	Crude ^a	=<3 months			
		>3 months			
	Adjusted model ^{a,b}	=<3 months			
		>3 months			
Adjusted model ^{a,c}	=<3 months				
	>3 months				
Adjusted model ^{a,d}	=<3 months				
	>3 months				



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Second	Crude ^a	=<3 months	>3 months
	Adjusted model ^{a,b}	=<3 months	>3 months
	Adjusted model ^{a,c}	=<3 months	>3 months
	Adjusted model ^{a,d}	=<3 months	>3 months
Third (if possible)	Crude ^a	=<3 months	>3 months
	Adjusted model ^{a,b}	=<3 months	>3 months
	Adjusted model ^{a,c}	=<3 months	>3 months
	Adjusted model ^{a,d}	=<3 months	>3 months

^a Study site as fixed effect in the model.

^b Adjusted for age, sex, chronic conditions and onset time.

^c Adjusted for age, sex, chronic conditions, onset time and GP visits

^d Adjusted for age, sex, chronic conditions, onset time, GP visits and hospitalisations

These results will also be displayed in graphical format.

We will use the Dersimonion Laird test to determine if the differences between the VE estimates of the different influenza phases are statistically significant.

The results will be presented by influenza A subtype and for influenza B, as well as for overall and target group for vaccination.

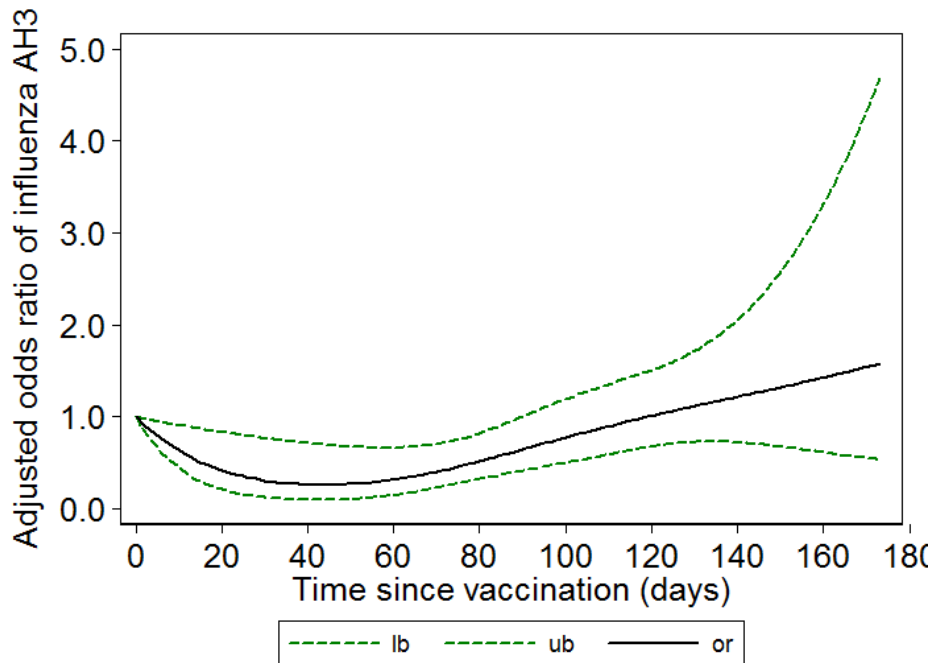
If sample size allows, tables will be presented by age groups (<75 years and >74 years) as well.

[Analysis Modelling Time since vaccination as a continuous variable](#)

In a second step, time since vaccination will be modelled using a spline, as outlined in the first section of Annex 8. We will provide giving a graphical output similar to the below. 95% CI along the modelled OR will be presented.



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In addition the information will be displayed in tabular format:

Delay between vaccination and onset of symptoms	Adjusted OR	Lower 95% CI	Upper 95% CI
0			
2			
4			
Etc.			

This analysis will be repeated also for each phase of the influenza season (first/second).

The results will be presented by influenza A subtype and for lineage of influenza B (if sample size allows), as well as for overall and target group for vaccination.

If sample size allows, tables will be presented by age groups (<75 years and >74 years) as well.



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Annex 9: Study-specific annexes

- Study specifications for each country are summarised in the annexes. Each study annex should include:
- description of the GPs participating in the study (number, distribution, catchment population, mode of recruitment);
- definition of beginning, peak, end of influenza season;
- ILI cases: specify if all ILI cases are recruited or a simple random or systematic sample is taken;
- seasonal and pandemic (if applicable) vaccines used;
- vaccine ascertainment method;
- information on application of ICD or ICHPPC-2 codes;
- sample size calculation;
- details on methods for data collection, data entry and data transmission;
- data validation procedures;
- laboratory issues (laboratory performing tests; tests used: PCR, culture, strain characterisation; methods for specimen collection, storage, transport; selection procedures for strain characterisation);
- consent, ethical procedures (oral/written consent; submission to ethics committee, if applicable);
- human resources needed;
- provisions to train GPs.