I-MOVE

Protocol for hospital-based, test-negative case–control studies to measure seasonal influenza vaccine effectiveness against influenza laboratory-confirmed SARI hospitalisation among the elderly across the European Union and European Economic Area Member States

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Abbreviations

EEA	European Economic Area
ECDC	European Centre for Disease Prevention and Control
EU	European Union
GP	General practitioner
ICD	International classification of diseases
ILI	Influenza-like illness
I-MOVE	Influenza monitoring vaccine effectiveness
IVE	Influenza vaccine effectiveness
MS	Member States
OR	Odds ratio
RT- PCR	Real time polymerase chain reaction
SARI	Severe acute respiratory infection
VC	Vaccination coverage
VE	Vaccine effectiveness

1 Background

In 2009 the European Council of Ministers recommended that all EU Member States (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 conditions.(1)

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 re-vaccination 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 sub-groups (age-groups, risk groups) and differs for the various influenza type and subtype outcomes measured.

Influenza VE (IVE) 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 data.(2)

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-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 continues to provide early and final IVE estimates in the elderly population to the World Health Organization (WHO) to complement virological information used to select the strains included in the vaccines.

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 influenza seasons.(3–6) 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 IVE and with a sufficient sample size to respond to these questions are needed.

Importantly, in the event of an influenza pandemic, having an established EU platform to rapidly measure IVE by vaccine type and product already in place will allow the rapid evaluation of any pandemic vaccine and adaptation of preventive and control strategies.

I-MOVE (Influenza Monitoring Vaccine Effectiveness in Europe), first established in 2007,(9) was the first network to monitor influenza vaccine effectiveness within and across the seasons in the EU and the European Economic Area (EEA). The network was initially 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 and laboratories from the EU and EEA. In 2010, EpiConcept initiated the InNHOVE (Influenza Network of Hospitals for Vaccine Effectiveness) project, aiming at measuring VE against hospitalised severe influenza among the population targeted by seasonal influenza vaccination. The project was run over three seasons (2011 through 2014) and involved up to five different study sites and a maximum of 24 hospitals.(10,11)

Building on these networks, the next stage was the development of the I-MOVE+ platform, which increased the number of participating hospitals to achieve sufficient sample sizes for the study questions listed above. The I-MOVE+ study targeted 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.

At the end of the 2017–18 season, there was no further funding provided by ECDC for the I-MOVE+ project into the 2018–19 season. Due to the importance of maintaining this hospital network, which will be particularly needed if there is a pandemic, EpiConcept has stepped in to provide funding for the 2018–19 season.

The I-MOVE hospital study estimates IVE in hospitalised elderly SARI patients, a patient group which is more likely to experience chronic co-morbidity due to age, hence are likely to be on statins (a class of drug used mainly to lower cholesterol). Studies have shown that, on the one hand, statin use reduces severity of influenza,(12) while on the other, statin use reduces IVE.(13) From the start of the 2018–19 season onwards, potential effect modification and/or confounding effects of statins on IVE will also be investigated.

Eight study sites involving 18 hospitals in seven EU MS will be included in the hospital study. The laboratory component of the network will include regional and national reference centres from the participating countries. The integration of virological data will be essential to interpret IVE 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. While each of the study sites can analyse their data separately, pooling the data into one analysis will provide a sample size big enough to answer study questions with reasonable precision.

The study sites will carry out case–control studies, based on the test negative design (TND), which is the main design used in IVE studies and is recommended by the EMA.(2,9,14)

This publication presents the core European protocol for the hospital-based study component of I-MOVE for the 2018–19 season, 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

2.1 Primary objective

The primary objective will be to measure, in EU/EEA MS, seasonal IVE against laboratory-confirmed influenza in elderly hospitalised SARI patients.

2.2 Secondary objectives

- To estimate seasonal IVE against laboratory-confirmed influenza requiring hospitalisation in elderly SARI patients:
  - in each of the participating study sites
  - by risk group (e.g. specific chronic conditions)
  - by age group (65–79 years, 80+ years)
- To estimate the effect of statins on laboratory-confirmed influenza in elderly SARI patients requiring hospitalisation
- To identify vaccine types (e.g. adjuvanted vs. non-adjuvanted, groups of vaccines (split virion, subunit, adjuvanted, trivalent vs. quadrivalent)) and brands with different effectiveness
- To understand the factors affecting IVE: duration of protection, the role of repeated seasonal vaccinations, the role of statins
- To identify key influenza virus phenotypic or genotypic evolutions that could affect vaccine performances and estimate VE against specific clades.

➢ *Each study site to specify the secondary objectives of their study*

3 Methods

3.1 Study design

- At study site level: hospital-based TND case–control study in each participating hospital
- At EU/EEA level: multicentre hospital-based TND case–control study in several countries/regions

3.2 Study population

The study population consists of all community-dwelling individuals aged 65 years and above hospitalised with SARI to one of the participating hospitals/services, with no contra-indication for influenza vaccination.

➢ *Each study site to specify the study population*

3.3 Study period

Seasonal influenza vaccine: The study will begin when the seasonal influenza vaccine of the corresponding season becomes available and the influenza season begins in the country/region and will finish at the end of the influenza period.

- *Each study site to specify the study period the definition of the beginning, peak and end of the influenza period at the study site according to the information provided by the country influenza surveillance system*

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 outcome of interest will be laboratory-confirmed influenza in patients hospitalised with a SARI and aged 65 years and above.

More specifically, they will be:

- 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 Case definition

3.5.1 SARI patient

A SARI patient will be defined as a hospitalised person with:

- at least one systemic symptom or sign: fever or feverishness, malaise, headache or myalgia or deterioration of general condition (asthenia or loss of weight or anorexia or confusion or dizziness)

AND

- at least one respiratory symptom or sign (cough, sore throat or shortness of breath)

at admission or within 48 hours after admission.

The symptoms should not have started (or clearly worsened, if chronic) more than 7 days before swabbing.

3.5.2 Hospitalised patient

A hospitalised patient will be defined as a patient who has been admitted in one of the participating hospitals during the study period, and has not been discharged home or home-equivalent before 24h.

3.5.3 SARI confirmed as Influenza (case)

An influenza case will be defined as a patient hospitalised with SARI with a respiratory sample positive for influenza.

3.5.4 SARI negative for Influenza (control)

A control will be defined as a patient hospitalised with SARI with a respiratory sample negative for influenza.

3.5.5 Exclusion criteria

The patient will not be enrolled in the study if she or he:

- is less than 65 years of age at the time of hospital admission
- has a contraindication for influenza vaccine
- had his/her SARI onset ≥ 48 hours after admission at the hospital
- has a history of hospitalisation within the 48 hours immediately prior to this admission
- is unwilling to participate or unable to communicate and give consent (the consent may also be given by her/his legal representative, or by specific consent procedures, acceptable according to the local ethical review process)
- is institutionalised at the time of symptoms onset (lives in a residence for people who require continual nursing care and have difficulty with the required activities of daily living)
- had a respiratory specimen taken ≥ 8 days after SARI onset
- tested positive for any influenza virus in the current season before the onset of symptoms leading to the current hospitalisation

Note: a patient can be selected several times as long as he/she does not have previous laboratory-confirmed influenza.

3.6 SARI patient identification – Algorithm for patient inclusion

Table 1: List of diagnosis codes for which patients could be screened for onset of SARI symptoms that started within the past 7 days, IMOVE hospital based IVE study.

Category	Morbidity	ICD-9	ICD-10
Influenza-like illness	Cough	786.2	R05
	Difficulty breathing	786.05	R06
	Sore throat	784.1	R07.0
	Dysphagia	787.20	R13
	Fever	780.6	R50.9
	Headache	784.0	R51
	Myalgia	729.1	M79.1
	Fatigue/malaise	780.79	R53.1, R53.81, R53.83
Cardiovascular diagnosis	Acute myocardial infarction or acute coronary syndrome	410-411, 413-414	I20-23, I24-25
	Heart failure	428 to 429.0	I50, I51
Respiratory diagnosis	Emphysema	492	J43.9
	Chronic obstructive pulmonary disease	496	J44.9

Category	Morbidity	ICD-9	ICD-10
	Asthma	493	J45
	Myalgia	729.1	M79.1
	Dyspnoea/respiratory abnormality	786.0	R06.0
	Respiratory abnormality	786.00	R06.9
	Shortness of breath	786.05	R06.02
	Other respiratory abnormalities	786.09	R06.00, R06.09, R06.3, R06.89
Infections	Pneumonia and influenza	480-488.1	J09-J18
	Other acute lower respiratory infections	466, 519.8	J20-J22
	Viral infection, unspecified	790.8	B34.9
	Bacterial infection, unspecified	041.9	A49.9
	Bronchitis	490, 491	J40, 41
Inflammation	SIRS* non-infectious without acute organ dysfunction	995.93	R65.10
	SIRS* non-infectious with acute organ dysfunction	995.94	R65.11
Diagnoses related to deterioration of general condition or functional status	General physical deterioration, lethargy, tiredness	780.79	R53.1, R53.81, R53.83
	Anorexia	783.0	R63.0
	Feeding difficulties	783.3	R63.3
	Abnormal weight loss	783.21	R63.4
	Other symptoms and signs concerning food and fluid intake	783.9	R63.8
	Disorientation/altered mental status	780.97	R41.0
	Dizziness and giddiness	780.4	R42
	Infective delirium	293.0, 293.1	F05
	Coma	780.01	R40.2
	Transient alteration of awareness	780.02	R40.4
	Other alteration of consciousness (somnolence, stupor)	780.09	R40.0, R40.1
	Febrile convulsions (simple), unspecified	780.31	R56.00
	Complex febrile convulsions	780.32	R56.01

**SIRS: Systemic inflammatory response syndrome*

SARI patients will be identified among patients hospitalised for at least 24 hours in one of the participating hospitals.

For hospitals with electronic patient records and/or diagnosis codes commonly displayed, SARI-related ICD codes will be sought. Patients admitted with any of the ICD codes listed in Table 1 will be approached; those meeting the SARI case definition and the inclusion criteria will be invited to be part of the study and sign informed consent (Figure 1).

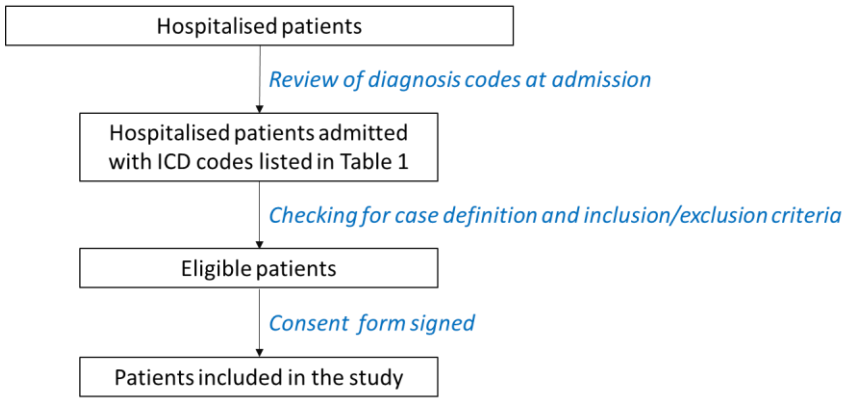

Figure 1: proposed inclusion algorithm for hospitals/services relying on common use of ICD codes, IMOVE hospital-based IVE study.

For hospitals where ICD codes at admission are not systematically collected or accessible, systematic screening of all patients admitted will be organised. This may be done by sensitisation of the medical staff at the beginning of the influenza season (Figure 2).

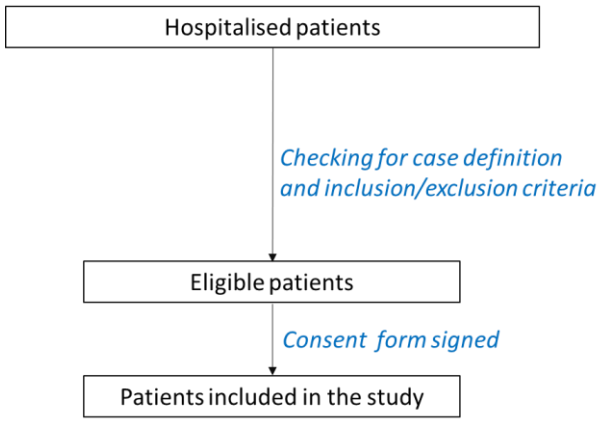

Figure 2: proposed inclusion algorithm for hospitals/services systematic screening of all admitted patients, IMOVE hospital-based IVE study.

Following the procedures outlined by each study (Figures 1 and 2), patients meeting the SARI case definition will be asked (directly or through their legal tutor) to provide consent and a nasal/throat respiratory specimen for influenza testing and to respond to an interview.

- *Each study site to describe procedures to identify study participants*

In case of budget limited to certain number of patients' inclusion, the study sites may need to switch from exhaustive to systematic sampling (e.g. inclusion of patients every second day). Systematic sampling procedures should be planned ahead by the study sites. During the period of systematic selection, the study sites will make sure to document the sampling fraction.

- *Study site foreseeing budget limits to detail the systematic sampling procedure*

3.7 Laboratory testing

Study nurses or physicians will collect respiratory specimens (see Section 4.4) from all eligible patients.

- *Each study site to describe the type and number of swabs taken by patient*

Influenza laboratory confirmation will be done using RT-PCR, multiplex RT-PCR and/or culture. Isolates will undergo molecular analysis for currently circulating influenza viruses.

Following the procedures outlined by each study, a systematic sample of isolates (or all isolates) will 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.

- *Each study site to describe the laboratory procedures (samples taken, storage, transport)*
- *Each study site to describe the tests used*
- *Each study site to describe if the laboratory participates in QA/QC (Quality Assurance/Quality Control) schemes*
- *Each study site to describe the selection of specimens and the procedures for genetic and antigenic characterisation (see Annex 4 for an example of results presentation)*

3.8 Exposure (vaccination)

3.8.1 Definition of vaccination status

Current seasonal influenza vaccine:

- An individual will be considered as vaccinated against influenza if s/he has received at least one dose of the influenza vaccine more than 14 days before SARI symptom onset.
- An individual will be considered as unvaccinated if s/he did not receive influenza vaccine in the current season of if s/he was vaccinated after onset of symptoms. (Anyone vaccinated ≤14 days before SARI symptom onset will be excluded from the primary analysis.)

Product-specific seasonal influenza vaccine:

- An individual is considered as vaccinated against influenza with a product-specific vaccine if s/he has received a vaccination with an influenza vaccine of a named product (see section "Vaccination status ascertainment") more than 14 days before symptom onset
- An individual is considered as unvaccinated if s/he did not receive influenza vaccine in the current season of if s/he was vaccinated after onset of symptoms. (Anyone vaccinated ≤14 days before SARI symptom onset will be excluded from analysis.).

3.8.2 Vaccination status ascertainment

The main exposure of interest in this study will be vaccination history with any influenza vaccine (seasonal or pandemic) in the season under investigation. The vaccination history includes date of administration, type of vaccine and brand name, and number of doses.

The sources of information for the vaccination status may include:

- vaccination registry
- consultation of the patient's vaccination card
- interview with the patient's GP
- interview with the patient's pharmacist
- data from the patient's insurance company showing evidence of pharmacy delivery or re-imbursement for influenza vaccine during the current influenza season.
- interview of the patient and/or his/her relatives

➢ *Each study site to describe how vaccination status ascertainment will be done*

3.8.3 Definition of statin status

➢ *Each site will define statin use based on data collected*

Statin status in current season:

- An individual will be considered as "on statin" if s/he has received at least one dose of statin on or before
  - seasonal influenza vaccination (if date of statin use available: for the analysis measuring the effect of statin on VE)
  - onset of symptoms (for unvaccinated individuals: for the analysis measuring the effect of statin on influenza)
  - 01 October of that season (so for 2018–19 season: 01 October 2018) if exact date of start of vaccine use is not available (or before start of vaccination campaign for countries with vaccination campaign starting later, e.g. Romania)
- An individual will be considered as "not on statin" if s/he did not receive statin
  - before the dates specified above in the protocol to document statin use

Product- or type-specific statin status:

- An individual is considered as being on a product- or type-specific statin if s/he has received a dose of such a product (see section "Statin status ascertainment") on or before
  - seasonal influenza vaccination (if date of statin use available: for the analysis measuring the effect of statin on VE)
  - onset of symptoms (for unvaccinated individuals: for the analysis measuring the effect of statin on influenza)
  - 01 October of that season (so for 2018–19 season: 01 October 2018) if exact date of start of vaccine use is not available (or before start of vaccination campaign for countries with vaccination campaign starting later, e.g. Romania)
- An individual is considered as not being on a product- or type-specific statin if s/he did not receive a dose of such a statin
  - before the dates specified above in the protocol to document product- or type-specific statin use

3.8.4 Statin use status ascertainment

Another exposure of interest in this study will be use of statins during the season under investigation. The statin history includes date the patient started on statins where known; else just the year, if the patient was known to have been on statins before the current season or if the precise date is unknown. If both of these are unknown, then a simple yes/no response to whether the patient was on statins at the start of October for that season will be used (e.g. on statins on 01 October 2018 for the 2018–19 season). In addition, statin history will include type of statin (synthetic vs natural) and brand name, and number/frequency of doses.

The sources of information for statin status may include:

- consultation of the patient's hospital record
- interview with the patient's GP
- interview with the patient's pharmacist
- data from the patient's insurance company showing evidence of pharmacy delivery or re-imbursement for statins during the current influenza season
- interview of the patient and/or his/her relatives

➢ *Each study site to describe how statin status ascertainment will be done*

3.9 Confounding factors and effect modifiers

3.9.1 Chronic diseases

List of underlying conditions which could be potential confounding factors/effect modifiers (for ICD codes see Table 2):

- anaemia
- chronic liver disease
- diabetes mellitus
- heart disease
- cancer
- immunodeficiency and organ transplant
- lung disease
- renal disease
- dementia
- stroke
- rheumatologic diseases
- obesity

➢ *Each study site to define the list of chronic diseases to be included and describe what the source of information will be*

3.9.2 Severity

The severity of an underlying condition is likely to be a stronger confounding factor than underlying condition alone. The severity of the underlying conditions will be measured by the number of hospital admissions due to underlying conditions in the 12 months prior to inclusion in the study.

3.9.3 Smoking history

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

3.9.4 Previous influenza and pneumococcal vaccinations

Vaccination against influenza in the last two seasons and vaccination against pneumococcal diseases will be collected.

The sources of information for vaccination in the last two seasons may include:

- vaccination registry
- consultation of the patient's vaccination card
- interview with the patient's GP
- interview with the patient's pharmacist
- data from the patient's insurance company showing evidence of pharmacy delivery or re-imbursement of influenza vaccine during the current influenza season.
- interview of the patient and/or his/her relatives

➢ *Each study site to describe how pneumococcal vaccination status is documented*

3.9.5 Functional impairment

Frailty may be associated with both vaccination and the risk to develop severe symptoms in case of influenza infection. We will capture the presence of functional impairment using a question related to the ability of patients to do a range of daily activities without assistance.

➢ *Each study site to include the definition used for impairment*

Table 2: ICD-9 and ICD-10 codes for chronic diseases.

Category	ICD-9	ICD-10	Underlying conditions included
Anaemia	280–285	D50-64	Nutritional anemias, Hemolytic anemias, Aplastic and other anemias and other bone marrow failure syndromes
Chronic liver disease	571	K70, K72-74, K754, K769	Alcoholic liver disease, Hepatic failure, Chronic hepatitis, Fibrosis and cirrhosis of liver, Other inflammatory liver diseases
Cardiovascular diseases	093, 112.81, 130.3, 391, 393–398, 402, 404, 410–429, 745, 746, 747.1, 747.49, 759.82, 785.2-3	A52.01, B37.6, B58.81, I05-9, I11, I13, I20-25, I26.09, I26.9, I27, I30-51, I97.0-1, R00.1, T81.718A, T81.72XA, T82.817A, T82.818A, Q20-24, Q25.1-2, Q26.0-1, Q26.8, Q87.4, R01.1-2	Syphilitic aneurysm of aorta, Candidal endocarditis, Toxoplasma myocarditis, Chronic rheumatic heart diseases, Ischemic heart diseases, Hypertensive heart and chronic kidney disease, pulmonary embolism with acute cor pulmonale, pulmonary heart diseases, diseases of pulmonary vessels, Other forms of heart disease (including Nonrheumatic valve disorders, pericarditis, endocarditis, myocarditis, cariomyophathy, heart failure, block, cardiac arrhythmias, heart failure), Complication of other artery / vein following a procedure, Embolism of cardiac/vascular prosthetic devices, implants and grafts, congenital malformations of cardiac chambers and connections or heart, Coarctation or atresia of aorta, Congenital malformations of great veins, Marfan's syndrome, Cardiac murmur
Diabetes	250	E10-11	Type 1 and Type 2 diabetes mellitus
Obesity	27800, 278.01, 278.03	E66.01, E66.2, E66.9	Obesity
Immunodeficiency* or organ transplant	042, 279, V08, V42	B20, D80-84, D89.8-9, Z21, Z94	HIV, immune deficiency, organ or tissue replaced by transplant
Renal disease	274.1, 408, 580–591, 593.71–593.73, 593.9	M10.30, N00-19, N20.0, N28.9	Gout due to renal impairment, Glomerular diseases, Renal tubulo-interstitial diseases, Acute kidney failure and chronic kidney disease, Calculus of kidney, Disorder of kidney and ureter, unspecified
Dementia	290, 294, 331	F01, F03, F05, G30, G31, G91, G94	Vascular dementia, other dementia, Delirium due to known physiological condition, Alzheimer's disease, Other degenerative diseases of nervous system
Stroke	348, 438	G93, I67.83, I69	Brain disorders, Posterior reversible encephalopathy syndrome, Sequelae of cerebrovascular disease

Rheumatologic diseases	446, 710, 714	M30-34, M35.0, M35.5, M35.8-9, M05-06, M08, M12.00	Polyarteritis nodosa and related conditions, Other necrotizing vasculopathies, Systemic lupus erythematosus (SLE), Dermatopolymyositis, Systemic sclerosis, Sicca syndrome, Multifocal fibrosclerosis, other systemic involvement of connective tissue, Rheumatoid arthritis with rheumatoid factor, Other rheumatoid arthritis, Juvenile arthritis, Chronic postrheumatic arthropathy
Cancer	140–208	C00-96	Malignant neoplasms and neuroendocrine tumours
Lung disease	011, 490–511, 512.8, 513–517, 518.3, 518.8, 519.9, 714.81	A15, J40–47, J60–94, J96, J99, J182,	Respiratory tuberculosis, Bronchitis, not specified as acute or chronic, Chronic bronchitis, Emphysema, Other chronic obstructive pulmonary disease, Asthma, Bronchiectasis, Hypersensitivity pneumonitis due to organic dust, Pneumoconiosis, Airway disease due to specific organic dust, Hypersensitivity pneumonitis due to organic dust, Respiratory conditions due to inhalation of chemicals, gases, fumes and vapor, Pneumonitis due to solids and liquids, Respiratory conditions due to other external agents, Acute respiratory distress syndrome, Pulmonary edema, Pulmonary eosinophilia, not elsewhere classified, Other interstitial pulmonary diseases, Abscess of lung and mediastinum, Pyothorax, Pleural effusion, Pneumothorax and air leak, Other pleural conditions, Intraoperative and postprocedural complications and disorders of respiratory system, not elsewhere classified, Other diseases of the respiratory system, Hypostatic pneumonia, unspecified organism,
		M34.81, M05.10	**Systemic sclerosis with lung involvement, Rheumatoid lung disease with rheumatoid arthritis**

*Note: Patients who are only treated with glucocorticoids and have no other immune deficiency, are considered immune suppressed when treated with high-dose corticosteroids (≥ 20 mg/day of prednisone or equivalent for ≥2 weeks) in the last 3 months.

3.9.6 Use of statins

Statins are a class of drugs primarily used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase. *In vitro* studies have shown that statin treatment reduces replication of the influenza viruses.(15) They also have immunomodulatory effects (suppressing T-cell activation) and anti-inflammatory effects (by inhibiting production of pro-inflammatory cytokines). Influenza is known to be a trigger for severe cardiovascular disease (CVD) outcomes,(16,17) and statins are used to reduce early morbidity and mortality from CVD.(18) Often, in the early course of critical influenza illness, pro-inflammatory cytokines are elevated. Hence a drug which inhibits production of these cytokines may also reduce severe influenza outcomes.(19) In the US, statins have been shown to reduce 30-day mortality by 41% in patients hospitalised with laboratory-confirmed influenza.(18) In addition, mouse models have shown the potential positive effect of statins on the outcomes of sepsis and acute lung injury.(20,21) However, in the UK, no benefit was shown for statin use in reducing respiratory disease incidence for persons aged 45 years.(22)

It has been shown that statin users have a weaker response to the influenza vaccine than non-users in persons hospitalised for SARI.(22) The authors of this study, however, stressed that it was not clear whether this was actually due to the statins or the reason for which those patients take statins, i.e. CVD co-morbidity. In this particular article, authors called for further studies examining the effect of statin use on influenza VE (IVE) for laboratory-confirmed influenza rather than all SARI. A recent study from the US which investigated the health insurance records of 2.8 million influenza-vaccinated statin users and non-users aged 65 years and over did not find a strong effect of statin use (given around the time of vaccination) on subsequent influenza-related GP or hospital visits.(23)

The I-MOVE hospital study estimates IVE in hospitalised elderly SARI patients. This particular patient group are more likely to have chronic co-morbidities due to their age, hence are likely to be on statins. To investigate potential effect modification and/or confounding effects of statins on IVE, we will collect information on the use of statin (including, where feasible the dosage and the type of statins) from the 2018–19 season onwards.

- *Each study site to describe how statin use is collected and ascertained*

3.9.7 Antiviral administration

The use of antivirals prior to swabbing may lead to misclassification biases. We will run sensitivity analyses excluding patients who were administered antivirals prior to swabbing. We will document whether the patients received any antiviral treatment in the 2 weeks preceding symptom onset and the type (curative or preventive) of antivirals received.

- *Each study site to list any antivirals administered*

3.9.8 Other respiratory viruses

Controls admitted with underlying lung diseases may be included due to an exacerbation of underlying conditions unrelated to SARI. Due to their underlying conditions, these patients will be more likely to be vaccinated than the source population. We may therefore overestimate the vaccine coverage in

the control group, hence overestimate the IVE. We will try to document the presence of respiratory infection among patients testing negative for influenza.

- *Each study site to list the other respiratory infection viruses tested for and included*

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.

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- *Each study site to specify the minimum sample size calculation*

In IVE estimation, sample size estimation is different from sample size estimation in hypothesis testing. Rather than being concerned about a VE estimation to cross 0% or not, we are more concerned with the precision around the estimate. For example, if we have an IVE of 70%, a lower boundary confidence interval of 1% does not provide us with a very informative VE estimate, even if the confidence interval does not include 0%. We are more concerned to have a VE estimate that is precise around the point estimate of 70% (e.g. with a lower boundary of 50%). The precision around the estimate is more informative than whether the confidence intervals include 0% or not. Indeed, if we have low VE estimate, which can be the case in particular for A(H3N2),(24–26) we would need a huge sample size to provide a VE estimate that does not include 0%. For example, if the true VE is 5–10%, then a study providing a lower boundary not including 0% would be unreasonably large.

The following sample size estimates focus on precision of the VE estimate (Table 3). As the lower confidence interval boundary is always larger than the upper confidence interval boundary we focus on a precision of the lower confidence interval, ranging between 10 and 30%. We also assume a case to control ratio of 1:1. We include varying vaccine coverage among the source population between 30% and 50%, varying vaccine effectiveness (with the OR between 0.2 and 0.7).

Table 3: Sample size calculations

Precision to lower CI boundary	Controls/ case	Detectable OR	Vaccine coverage in source population/ controls	Number of cases	Number of controls	VE	CI
0.3	1	0.2	0.3	85	85	80	51–92
0.3	1	0.3	0.3	118	118	70	40–85
0.3	1	0.4	0.3	157	157	60	30–77
0.3	1	0.5	0.3	203	203	50	20–69
0.3	1	0.6	0.3	255	255	40	10–60
0.3	1	0.7	0.3	314	314	30	0–51

0.2	1	0.2	0.3	148	148	80	60–90
0.2	1	0.3	0.3	216	216	70	50–82
0.2	1	0.4	0.3	299	299	60	40–73
0.2	1	0.5	0.3	395	395	50	30–64
0.2	1	0.6	0.3	507	507	40	20–55
0.2	1	0.7	0.3	633	633	30	10–46
0.1	1	0.2	0.3	433	433	80	70–87
0.1	1	0.3	0.3	681	681	70	60–77
0.1	1	0.4	0.3	985	985	60	50–68
0.1	1	0.5	0.3	1346	1346	50	40–58
0.1	1	0.6	0.3	1764	1764	40	30–49
0.1	1	0.7	0.3	2240	2240	30	20–39
0.3	1	0.2	0.4	63	63	80	49–92
0.3	1	0.3	0.4	91	91	70	40–85
0.3	1	0.4	0.4	125	125	60	30–77
0.3	1	0.5	0.4	165	165	50	20–69
0.3	1	0.6	0.4	212	212	40	10–60
0.3	1	0.7	0.4	265	265	30	0–51
0.2	1	0.2	0.4	111	111	80	60–90
0.2	1	0.3	0.4	168	168	70	50–82
0.2	1	0.4	0.4	238	238	60	40–73
0.2	1	0.5	0.4	323	323	50	30–64
0.2	1	0.6	0.4	421	421	40	20–55
0.2	1	0.7	0.4	534	534	30	10–46
0.1	1	0.2	0.4	323	323	80	70–87
0.1	1	0.3	0.4	528	528	70	60–77
0.1	1	0.4	0.4	786	786	60	50–68
0.1	1	0.5	0.4	1098	1098	50	40–58
0.1	1	0.6	0.4	1466	1466	40	30–49
0.1	1	0.7	0.4	1891	1891	30	20–39
0.3	1	0.2	0.5	51	51	80	51–92
0.3	1	0.3	0.5	77	77	70	40–85
0.3	1	0.4	0.5	109	109	60	30–77
0.3	1	0.5	0.5	148	148	50	20–69
0.3	1	0.6	0.5	193	193	40	10–60
0.3	1	0.7	0.5	246	246	30	0–51
0.2	1	0.2	0.5	90	90	80	60–90
0.2	1	0.3	0.5	142	142	70	50–82
0.2	1	0.4	0.5	208	208	60	40–73
0.2	1	0.5	0.5	289	289	50	30–64
0.2	1	0.6	0.5	384	384	40	20–55
0.2	1	0.7	0.5	495	495	30	10–46
0.1	1	0.2	0.5	262	262	80	70–87
0.1	1	0.3	0.5	447	447	70	60–78
0.1	1	0.4	0.5	687	687	60	50–68
0.1	1	0.5	0.5	983	983	50	40–58

0.1	1	0.6	0.5	1337	1337	40	30–49
0.1	1	0.7	0.5	1751	1751	30	20–39

The sample size estimates above are for the crude analysis and an adjusted analysis would require a higher sample size.

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

See also the Analysis section on sample size requirements for analyses.

3.11 Data

3.11.1 Sources of information

Data will be collected using a standardised questionnaire/data collection form. The source(s) of data may include:

- hospital medical records
- interview with patient or his/her family
- interview with patient's GP
- interview with patent's pharmacist
- vaccination register
- laboratory

➢ *Each study site to define the sources of information used for each variable collected*

➢ *Each study site to specify the hypothesis used for sample size calculation*

3.11.2 Collected information

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

- study identification: country, hospital, first ward of referral
- demographics
- SARI signs, symptoms
- date of onset of SARI
- date of admission, of swabbing
- laboratory results (including information antigenic and genetic analysis, where available)
- underlying chronic conditions
- number of hospitalisations for the chronic diseases in the previous 12 months
- number of GP visits in the previous 3 months
- use of statins
- smoking history
- other respiratory viruses
- pandemic vaccination including number of doses, date, product (if applicable)
- current season influenza vaccination including date and product
- influenza vaccination in the two previous seasons (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 becomes available.

3.11.3 Data entry validation

For hospitals using electronic medical records, if paper questionnaires are used, a sample of them will be checked against the medical records and against the study database. The agreement between patient vaccine records/vaccination status reported by study participant/vaccine registries will be measured when vaccination registries are available.

- *Each study site to specify how data are validated*

3.12 Data management

3.12.1 Individual analysis

Web-based data collection methods or paper-based methods can be used. Data entry will include checks to minimise data entry errors. Double data entry is recommended unless electronic medical records are used.

Laboratory information will be reported to the study site coordinator using the reporting procedures existing in each study site for influenza surveillance.

For the multi-centre pooled analysis, study sites will send an anonymised database to the coordinating team.

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.

- *Study teams to specify procedures of data management and procedures to comply with the RGDP requirements*
- *Study teams to provide a codebook that includes the variable names, variable descriptions, and the coding of variable values.*

3.12.2 Data cleaning

Summary and frequency tables as well as visual representations 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 respiratory specimen collection before date of onset of symptoms). Ideally, these checks will be included in the electronic questionnaire in order to avoid inconsistencies in the data entry. These values will be checked against the questionnaires or queried with the hospitals. Any changes to the data will be documented and stored separately from the crude database. Any recoding of data (e.g. age) will be documented. A guide and/or an example Stata do-file for data cleaning will be provided if so desired.

- *Study teams to specify the data checking and cleaning process*

3.12.3 Management database for pooled analysis

The coordinating team will conduct the pooled analysis. Data validation, cleaning and verification will be carried out at study level. Each individual study database will be sent to the coordinating team study database using a secure protocol (see Annex 2: Dataflow for pooled database). All personal identifier information such as names, addresses, and medical registration codes will be deleted before data transmission to the coordinating team, where all individual data will be pooled.

A country (or study) identifier will be included in each record (e.g. ES for Spain, UK for the United Kingdom), a hospital code will be included (e.g. a unique number), and each record will be given a unique number. This number will also be included in the study team's database and will be used by the coordinating team and the study teams during pooling, so that records can be traced back whilst maintaining anonymity, if there should be any further queries. Tracing back will be performed by the study teams, not by the coordinating team. Study databases can be sent in any format.

Summary and frequency tables 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 respiratory specimen collection before date of onset of symptoms). Any improbable, illegal or missing values will be reported to the study site in question.

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.

A study-site specific flowchart of exclusions and restrictions will be shared with each of the study sites. Variables will be recoded and new variables generated. The recoded data will be stored separately from the crude data and recoding will be documented.

3.12.4 Missing data

Any missing data will be described. If many data are missing and there is no evidence of bias in the missing data, and variables that are considered good predictors of the missing data are available, multiple imputation methods at study level will be used to replace missing values. A sensitivity analysis will be carried out comparing results from the complete case analysis (where records with missing data will be dropped) and the full set analysis (with imputed data).

3.13 Data Analysis

The analysis will be carried out first for each individual study site. In a second step, the pooled analysis will be conducted (see Annex 3).

3.13.1 Individual study analysis

Descriptive and univariable analysis

The proportion of eligible hospitalised cases and controls who accepted to participate in the study will be calculated. Reasons for no participation will be documented. Study participants will be described by baseline characteristics. Baseline characteristics of cases and controls will be 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 will be measured for both case and control groups.

Measure of effect

Vaccine effectiveness will be computed as VE = 1 – OR (expressed as a percentage). A 95% confidence interval will be computed around the point estimate. (For studies using the screening method, a generic protocol is available upon request.)

Stratified analysis

The analysis will be stratified according to (depending on sample size):

- age groups 65–79 years, 80+ years
- absence, presence of at least one, presence of more than on high-risk condition
- specific chronic conditions (e.g. respiratory, cardiovascular diseases)
- statin use
- time: early influenza season, peak, late influenza season
- vaccine type (adjuvanted vs non adjuvanted)
- vaccine brand

Virus type-/subtype-/lineage-specific outcomes will be used.

A sufficient sample size should be planned in order to ensure enough individuals in each stratum for a precise estimate. Effect modification will be assessed comparing the OR across the strata of the potential effect modifiers. Confounding will be assessed by comparing crude and adjusted OR for each potential confounder.

Multivariable analysis

A multivariable logistic regression analysis will be conducted to control for negative and positive confounding. Odds ratios and standard errors will be obtained. Variables will be tested for multicollinearity. Interactions will be tested using the likelihood ratio test or Wald's test and will be included in the model if significant at the 5 % level. Factors other than statistical significance (prevalence of exposure, magnitude of OR) will also be used as criteria for inclusion of a variable or an interaction term. 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, GP visits and hospitalisations in the past 12 months. 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 Stata programme "mkspline" can 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 one illustrated by Table 4 can be used (variables presented just as example of the output format). Useful information includes numbers of cases and controls (overall and vaccinated) and presentation of results for different models.

Table 4. Influenza vaccine effectiveness against influenza A(H1N1)pdm09 adjusted for various covariables by age group, hospital-based influenza vaccine effectiveness study, IMOVE, 20xx–yy.

Type/subtype	Population included	Analysis scenarios/adjustments made	VE (%)	(95%CI)
A(H1N1)pdm09	All ages	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 *		
	<80 years	N (cases/ vaccinated; controls/ vaccinated)		
		Crude*		
		Adjusted for onset month, age (cubic spline), chronic condition*		
	>79 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.

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 7 for further information on VE by time since vaccination)
- VE of previous season influenza vaccination only, current season influenza only and combined season vaccination
- As a sensitivity analysis, VE will be calculated:
  - considering those vaccinated <15 days before onset of symptoms as unvaccinated (in the main analysis these records will be excluded)
  - using, as a control group, only controls testing positive for at least one non-influenza respiratory virus

Use of propensity scores

To limit the number of co-variables to include in the multivariable model, **if sample size allows**,

we will build and try to adjust our estimates on propensity scores. Propensity scores can be defined as the conditional probability of receiving the vaccine given a number of observed covariables.

In propensity score matching, a propensity score for vaccination is calculated for cases and controls. Cases and controls are then matched by propensity score and all non-matched patients are discarded. Variables used to calculate the propensity score will include variables related to the vaccination and outcome. Care will be taken to avoid correlation and overmatching.

The user-written "psmatch2" routine can be used in Stata.

Controlling for hospital effect

Primary analysis will be carried out using simple logistic regression to obtain the individual study estimates. However, there could be an effect of the hospital 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 hospital as a random effect will be carried out.

Minimum sample size

Sample sizes may be very small for some sub-analyses. Different criteria can be used to determine whether the sample size is large enough to obtain a valid measure of IVE:

- There are at least 10–15 cases (or controls, whichever is smaller) in the sub-analysis for crude analyses and more for adjusted analyses (e.g. at least 10 for each parameter in the model)
- 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% (uninformative).

With low sample size, sensitivity analyses can be carried out using penalised logistic regression.

- *Each study site to specify criteria used to determine minimum sample size if desired.*

3.13.2 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, RO for Romania), a hospital code is included, 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 3 for detailed guidelines to the pooled analysis. For the pooled data, interim analyses will be conducted in different periods according to the available sample size.

The timing to conduct each interim analysis will depend on the time needed to reach the appropriate sample size. This will depend mainly on the incidence of hospitalisation, influenza incidence, vaccination coverage, the recruitment strategy within hospitals and the number of participating hospitals/services per hospital. The sample size for brand VE will depend on the vaccination coverage for each brand and therefore on the market share.

3.14 Potential biases

3.14.1 Negative confounding

Negative confounding refers to biases that reflect the fact that high-risk groups (people more likely to develop severe complications) will be more likely to be vaccinated and therefore reduce IVE. If negative confounding is present, the VE will be underestimated. Adjustment for potential negative confounding factors documented in the study (e.g. presence of chronic diseases) will minimise negative confounding.

3.14.2 Positive confounding

Positive confounding refers to biases that reflect a 'healthy vaccine effect'. People with a healthy lifestyle will be more likely to accept vaccination, thus leading to an increase of measured VE. Or, similarly, people being in a state of "extreme frailty" will not be offered vaccination. If positive confounding is present, VE will be overestimated.

Positive and negative confounding will be minimised through stratification and multivariable analysis. It will not be 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. Therefore, any residual positive or negative confounding may remain.

3.14.3 Representativeness of subjects included in the study

The study includes only cases that are hospitalised. Health-seeking behaviour may differ by country depending on the case management strategy (e.g. recommendation of seeing a GP first). In some cases, the management strategy will have an impact on the delay between onset of symptoms and hospitalisation. This, in turn, may have an impact on the time lag between onset and respiratory specimen collection, and may affect positivity rates between study sites. Beside the collection of dates of onset/admission/respiratory specimen collection, health-seeking behaviour and case-management strategies should be described for each study and it should be noted how these may affect the VE estimates.

- *Each study site to describe the potential limitations in terms of representativeness of the subjects included*

3.14.4 Validation of exposure

The vaccination status is the exposure of interest and the validity of vaccination data should therefore be checked carefully. If the vaccination status is reported by the patient only without

further proof, information bias may occur. We will validate the vaccination status of cases and controls using an independent source (i.e. vaccination register, GPs).

- *Each study site to describe how the source of exposure validation and its potential limitations*

3.14.5 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 will be low if the sample size per country is small. In this case, the test may not detect the presence of heterogeneity, even if present. It is important that heterogeneity will also be 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 Consent

Each study will comply with national ethics committee requirements. Informed consent will be required from all participants or legal tutors. The national ethics committees will specify whether oral or written consent will be required. Specific consent procedures may be needed for unconscious patients and patients with deterioration of general condition or functional status, unable to sign the consent (e.g. oral witnessed consent, consent by the next of kin, etc). A copy of the ethical approvals should be sent to the coordinating centre.

- *Each study site to describe the procedures to comply to the national ethics committee requirements and the type of informed consent needed as well as whether consent can be obtained for a legal tutor*
- *Each study site to send a copy of the ethical approval to the coordinating centre*

3.16 Dissemination of results

The enrolment of cases/controls will be regularly updated by each study coordinator on a website developed for the multicentre study. Initial IVE estimates (intra-seasonal) will be disseminated early during the influenza season; final estimates will follow at the end of the season.

3.16.1 Publications, scientific communication

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

The list of authors will respect the recommendations of authorship stated by the International Committee of Medical Journal Editors http://www.icmje.org/ethical_1author.html. The actual

authorship for the pooled article will be discussed and agreed with the study teams at the beginning of the study.

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.

3.17 Training

Investigators and data collectors will be trained on the study protocol before the start of the study. They will receive the protocol, questionnaires and laboratory respiratory specimen collection procedures.

- *Each study site to describe the trainings to be organised*

4 Logistical aspects

4.1 Study leader

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

4.2 Human resources

In each hospital/hospital network, an investigator will be in charge of monitoring data collection at the hospital level. Study investigators at the hospital will collect information among cases and controls. 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 will be organised by the coordinating team/study sites in order to carry out an appraisal of the ongoing studies in the various countries involved. The appraisal team will be composed of two persons from the various project partners.

4.4 Respiratory specimen collection

By default, the respiratory specimen will be collected through nasopharyngeal swabbing or concurrent nasal and oral swabbing. For patients unable to undertake such swabbing, other methods such as aspirate or nose blowing could be applied.

- *Each study site to describe the specimen collection procedures.*

4.5 Laboratory tests

High specificity is needed for influenza confirmation. Influenza laboratory confirmation will be done using RT-PCR, multiplex RT-PCR and/or culture.

- *Each study site to describe the tests and the kits used for influenza and, if needed, other respiratory virus detection*

- *Each study site to specify sequencing methods.*

Quality control tests should systematically be run using PCR to test for presence of cells in the respiratory specimens.

- *Each study site to describe quality controls for specimens*
- *Each study site to describe genetic and antigenic analyses.*

4.6 Computer support

Data collection and entry will be conducted at the country level. The coordinating team will provide a structured data entry form. For countries willing to submit data electronically, the coordinating team could provide services to develop an online questionnaire.

- *Each study site to describe the data collection tools used*

4.7 Standard operating procedures

Standard operating procedures should be used by investigators during all the steps of the study for identification of study subjects, data collection, laboratory methods, data entry, monitoring, etc.

- *Each study site to develop (or adapt pre-existing) study SOP to be used by the study team*

4.8 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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Annexes

Annex 1: List of variables, definitions and coding; IMOVE hospital based IVE study

	Variable	Type	Values and coding	Definition
General	idcountry	Numeric	Coded according to international country codes	Identifier uniquely identifying the country
	id	Numeric	Unique integer	Unique number for each patient
	hospitalcode	Numeric	Unique integer	Unique number for each hospital
	hospitalward	text		First ward of referral
	icu (optional)	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Admission to intensive care unit
	sex	Numeric (binary)	0 = female 1 = male	Sex of study participant
Dates	dob	Date	dd/mm/yyyy	Date of birth of study participant
	admisdate*	Date	dd/mm/yyyy	Date of hospital admission
	onsetdate*	Date	dd/mm/yyyy	Date of onset of symptoms
	swabdate *	Date	dd/mm/yyyy	Respiratory specimen collection date
	dischargedate (optional)	Date	dd/mm/yyyy	Date of hospital discharge
	deathdate	Date	dd/mm/yyyy	Date of death
Death	deathcause	Text		Cause of death
Admission symptoms	feverishness*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Subfebrility (37–38°C) (to construct SARI case definition)
	fever*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Fever (to construct SARI case definition)
	malaise*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Malaise (to construct SARI case definition)
	headache*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Headache (to construct SARI case definition)
	myalgia*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Myalgia (to construct SARI case definition)
	sorethroat*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sore throat (to construct SARI case definition)
	cough*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cough (to construct SARI case definition)
	suddenonset*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sudden onset
	short_breath*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Shortness of breath (to construct SARI case definition)
	general_deter*	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Deterioration of general condition (asthenia or loss of weight or anorexia or confusion or dizziness) (to construct SARI case definition)

	Variable	Type	Values and coding	Definition
Laboratory confirmation	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_yam	Numeric (categorical)	1 = Negative 2 = Positive 8 = Do not know	Laboratory result: B virus lineage Yamagata
	lineage_vic	Numeric (categorical)	1 = Negative 2 = Positive 8 = Do not know	Laboratory result: B virus lineage Victoria
	genetic_group	Text		Laboratory result: genetic group
	antigenic_analysis	Text		Laboratory result: antigenic group
Vaccination status current and past seasons	seasvaccany	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination in current season (season n)
	seasvaccbrand	Text		Vaccine brand
	seasvaccdate	Date	dd/mm/yyyy	Vaccination date
	seasvacc_n1	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received seasonal influenza vaccination in previous season (season n-1)
	seasvacc_n2	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received seasonal influenza vaccination in season n-2
Pneumococcal vaccination	ppv_vacc	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received PPV23 vaccination
	ppv_vaccdate	Date	dd/mm/yyyy	Date of last PPV23 vaccination
	pcv_vacc	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received PCV7/10 or 13 vaccination
	pcv_vaccdate	Date	dd/mm/yyyy	Date of last PCV7/10 or 13 vaccination

	Variable	Type	Values and coding	Definition
Underlying chronic conditions	liver_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic liver disease (excluding cancer)
	diabetes	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Diabetes
	heart_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Heart / cardiovascular disease
	cancer	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cancer
	immuno	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Immunodeficiency or organ transplant
	immuno_treatment (optional)	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Immunodeficiency due to treatment
	lungdis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Lung disease (excluding cancer)
	anemia	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Anemia
	ren_disease	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Renal disease (excluding cancer)
	dem	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Dementia
	stroke	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	History of stroke
	rheumat	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Rheumatologic diseases
	obese	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Obesity
	dependency	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Patient has difficulty doing at least one of these actions (see list[1])
	severity	Numeric (count)	integer	Number of hospitalisations previous 12 months for the chronic disease

[1]Actions are: Eating; moving from bed to chair and back; doing his/her personal toilet; getting on and off toilet; bathing him/herself; walking on level surface; ascend and descend stairs; dressing; controlling bowels; controlling bladder.

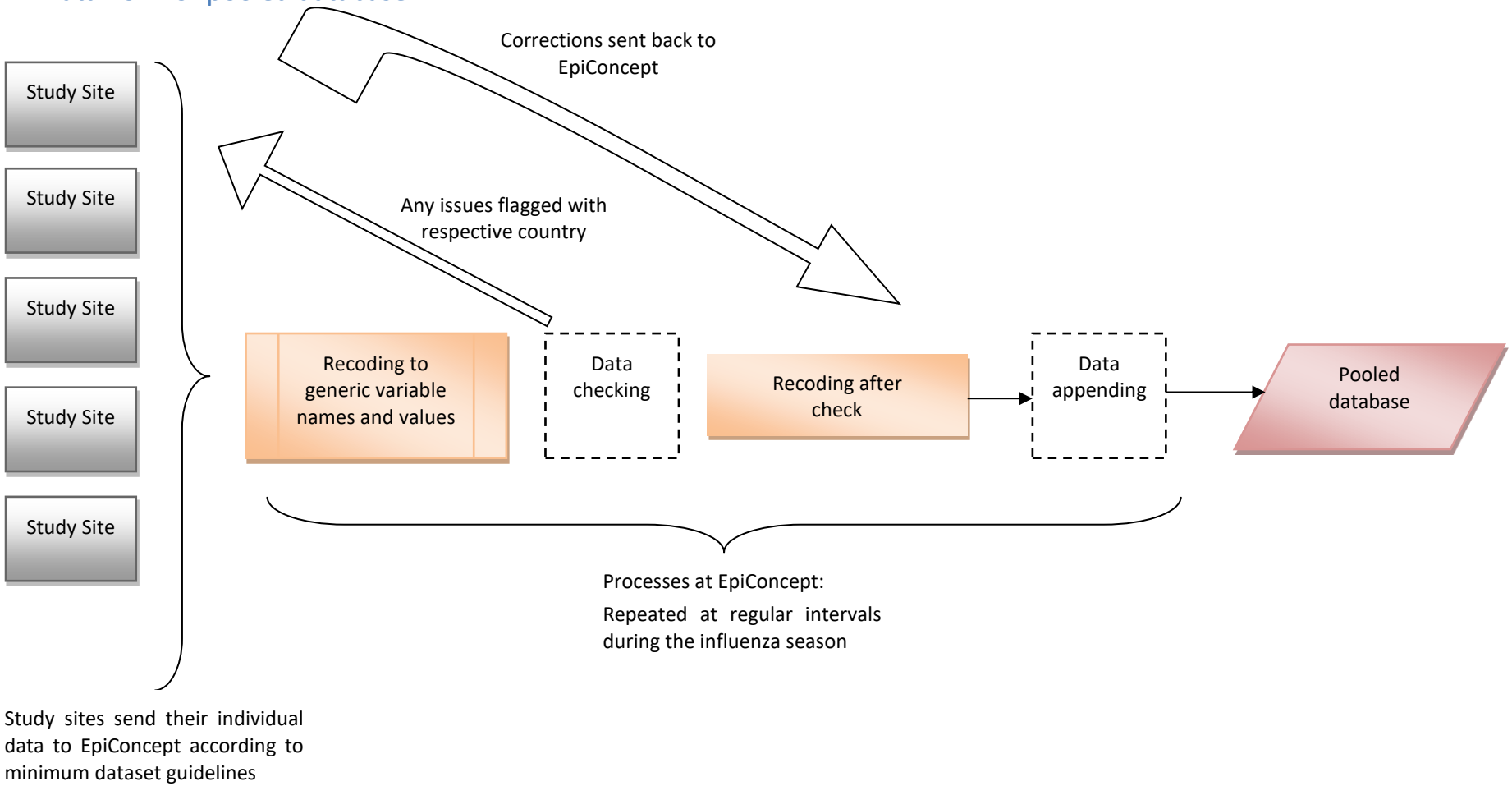
	Variable	Type	Values and coding	Definition
Use of statins	statin	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Patient was under statin treatment at any point during the season
	stat_brand	Text		Name of statin product used
	stat_type	Numeric (categorical)	1 = Synthetic 2 = Natural 8 = Do not know	Synthetic vs natural statin
	stat_dose_mg	Numeric (in mg)		Statin dose in atorvastatin equivalents (in mg)
	stat_dose_fr1	Numeric	0=per day 1= per week 8 = do not know	Frequency of statin dose (per day vs per week)
	stat_dose_fr2	Numeric	Integer	# times dose given per day or week
	stat_onsetd	Date	dd/mm/yyyy	Date patient started statin treatment[2]
	stat_onsety	Numeric	yyyy	Year; if patient started statins before this season or precise date (stat_onsetd) is NK
	stat_seas	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Patient was on statin on 01-oct-2018
	stat_presymp	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Patient started statin before symptom onset
	stat_prevacc	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Patient started statin before vaccination
Health care utilisation	gpvisit (optional)	Numeric (count)	integer	Number of GP consultations previous 3 months
Smoking	smoking	Numeric (categorical)	0 = Never 1 = Former 2 = Current 8 = Do not know	Never, former (stopped smoking at least 1 year before inclusion in the study), current smoker
Antivirals	antivir	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Has the patient received an antiviral treatment within the 2 weeks before swabbing?
	antivirtype (optional)	Text		Type of antiviral (brand name)
Other respiratory viruses (optional)	resp_virus	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Does the patient test positive for any non-influenza respiratory virus?
Exclusion criteria	pre_flu	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Previous lab-confirmed influenza in the current season
	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
	hosp_before	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Exclusion criteria: hospitalisation in the last 48 hours before SARI onset

[2]Note: if only the first date variable is provided (stat_onsetd), we can then calculate the remaining four statin date variables during analysis. If the first date is **not** available, however, we would then need the second (if the year is before the current season), or the third, and could then calculate the fourth and fifth. If neither the first, second nor third are available, or if the second is available and it is within the current season, then we do need to have the last three, or the last two variables provided.

In a pandemic, these additional variables may be required:

Variable name	Type	Values and coding	Definition
panvaccany	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received pandemic flu vaccination
panvaccdate1	Date	dd/mm/yyyy	Vaccination date first dose
panvaccdate2	Date	dd/mm/yyyy	Vaccination date second dose
panvacctype	Text		Type of vaccine (product name)
panvaccdose	Numeric	0, 1, 2	Number of doses received

Annex 2: Data flow for pooled database



Annex 3: Pooled data analysis

Descriptive analysis

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

- Number of hospitals participating and catchment population
- Beginning of the study
- Beginning of influenza period, peak, end
- Beginning of vaccination campaigns for seasonal vaccine
- Vaccines used
- Estimated vaccine coverage in the country/region by vaccine brand in the elderly
- Number of patients screened
- Number of patients excluded per reasons for exclusion

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, stratified by the following:

- age groups 65–79 years, 80+ years
- specific chronic conditions (e.g. respiratory, cardiovascular diseases)
    - absence, presence of at least one, presence of more than one high-risk condition
- time: early influenza season, peak, late influenza season
- 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
- vaccine brand
- previous vaccination
- presence/absence of statin use

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

Identifying heterogeneity, testing for heterogeneity

Study-specific crude and adjusted ORs and their confidence intervals 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 also be checked. 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 and should be excluded from the pooled analysis.

Statistical heterogeneity between studies will be tested using Q-test and the I^2 index (see boxes for formulae below). The Q statistic follows a Chi^2 distribution (with k-1 degrees of freedom). The Q-test reports presence or absence of heterogeneity, while the I^2 index (based on the Q-statistic) quantifies the

extent of the heterogeneity. According to the Higgens and Thompson classification, an I^2 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.

One-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 site as a fixed effect. 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.

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.

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.

Two-stage pooled analysis approach

If adequate sample size by study is achieved to obtain an adjusted OR, 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 (τ2). This will give the pooled odds ratio and standard error.

$$\log(OR_R) = \frac{\sum w_i^* \times \log(ORi)}{\sum w_i^*}$$

$$wi^* = \frac{1}{vi + \tau^2}$$

The study specific ORs and their confidence intervals, 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.

Stratified analysis

The same 2-stage analysis will be carried out for the following strata if sample size permits:

- age groups 65–79 years, 80+ years
- absence, presence of at least one, presence of more than on high-risk condition
- specific chronic conditions (e.g. respiratory, cardiovascular diseases)
- time: early influenza season, peak, late influenza season
- vaccine type (adjuvanted vs non adjuvanted)
- vaccine brand
- previous vaccination

Controlling for hospital effect

Primary analysis will be carried out using simple logistic regression to obtain the individual study estimates. However, there could be an effect of the hospital 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 hospital as a random effect will be carried out when using a 1-stage pooled analysis.

Multi-level logistic regression can also be carried out for each individual study with hospital 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 datasets will include age, time of onset of symptoms, number of hospitalisations in the previous 12 months and GP visits in the previous 3 months. 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 Stata programme “mkspline” will be used.

Annex 4: 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																

B(Yamagata)	Country	Region/City	ID number I-MOVE case–control study	Date sample	Strain										GISAID 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 5: Generated/recoded variables

Variable name	Type	Values and coding	Definition
cases	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for any influenza type
casea	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for any influenza A type
caseh1	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for influenza type A(H1N1)
caseh3	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for influenza type A(H3N2)
caseb	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for any influenza B type (regardless of lineage)
caseby	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed influenza B Yamagata lineage
casebv	Numeric (binary)	0 = No 1 = Yes	Indicates SARI case that is lab-confirmed for influenza B Victoria lineage
sari	Numeric (binary)	0 = No 1 = Yes	Variable that corresponds to EU SARI 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
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
anychron	Numeric (binary)	0 = No 1 = Yes	0 indicates no 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

Variable name	Type	Values and coding	Definition
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), hospitalised 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 = 65–79 years 1 = 80–max years	The continuous variable age visit is grouped into 2 age groups, used for stratification

Variable name	Type	Values and coding	Definition
onsetweek1	Continuous	Integer	Week of onset of SARI 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-adjuvated vaccine >14 days before symptom onset are coded as 2 and those unvaccinated or vaccinated <15 days before symptom onset are coded as 0.
vaccgroup	Numeric (categorical)	0 = Not vaccinated 1 = Inactivated trivalent subunit (egg propagated) 2 = Inactivated trivalent split virion (egg propagated) 3 = Adjuvanted 4 = Inactivated trivalent subunit (cell propagated) 4 = Inactivated trivalent subunit (cell propagated) 5 = Inactivated quadrivalent subunit (egg propagated)	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

Annex 6: Stata syntax (example)

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:

```
// 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

Annex 7: 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 =< 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 =< 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.

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 IVE 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 (*note: the proposed models are examples*):

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			
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.

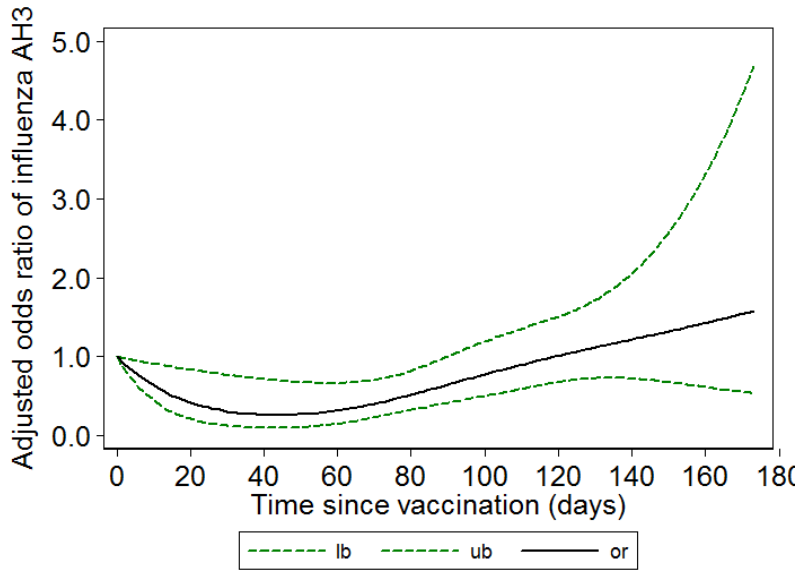
We will use the Dersimonion Laird test to determine if the differences between the VE estimates of the different influenza phases are statistically significant.

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 (<80 years and >79 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 this Annex. We will provide a graphical output similar to the below. 95% CI along the modelled OR will be presented.



In addition, the following 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 (<80 years and >79 years) as well.

Annex 8: Study-specific annexes

Study specifications for each country are summarised in the annexes. Each study annex should include:

- description of the hospitals participating in the study (wards involved, bed capacity, catchment population, detailed mode of recruitment including the use of computerised system to identify SARI patients);
- definition of beginning, peak, end of influenza season;
- seasonal and pandemic (if applicable) vaccines used;
- vaccine ascertainment method;
- 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);
- human resources needed;
- provisions to train hospitals.

Annex 9: History of changes to the generic protocol

The broad adaptation and use of this generic protocol led to identifying potential points of improvement. This paragraph aims at listing the changes brought to the protocol throughout its use. Changes are displayed in red text.

2015–16 to 2016–17 season

SARI case definition:

The definition of "deterioration of functional status was difficult to interpret and removing this symptom from the 2015-16 data led to excluding 1/1802 SARI cases only.
Study sites required a more specific definition of "deterioration of general condition".

2015–16 season:

(at least one systemic symptom or sign: fever or feverishness, malaise, headache or myalgia) OR deterioration of general condition OR deterioration of functional status) AND (at least one respiratory symptom or sign (cough, sore throat or shortness of breath)) at admission or within 48 hours after admission.

2016–17 season:

(at least one systemic symptom or sign: fever or feverishness, malaise, headache or myalgia or deterioration of general condition (asthenia or loss of weight or anorexia or confusion or dizziness)) AND (at least one respiratory symptom or sign (cough, sore throat or shortness of breath)) at admission or within 48 hours after admission.

Exclusion criteria

Comment during site visits: *"Repeated hospitalisation even within one week is surprisingly common in this target group".* To deal with patients hospitalised a few days before the SARI symptoms proceeding the current hospital stay, we added the following exclusion criteria:

was hospitalised < 48 hours prior to SARI onset

Study sites reported difficulties to define "institutionalised patients". This exclusion criteria further specified as:

is institutionalised at the time of symptoms onset (lives in a residence for people who require continual nursing care and have difficulty with the required activities of daily living)

Patient inclusion

Chronically ill patients are likely to be hospitalised more than once in a given season. To clarify how to deal with these patients, we included the following sentence in the protocol:

Note: a patient can be selected several times as long as he/she does not have a previous laboratory confirmed influenza

Study sites usually have limited budget to conduct this study. The magnitude and duration of influenza seasons cannot be forecasted and the sampling proposed here is exhaustive. To guide study sites in need to restrict sample size due to budgetary constraints, we added the following section:

In case of budget limited to certain number of patients' inclusion, the study sites may need to switch from an exhaustive to a systematic sampling (e.g. inclusion of patients every second day). A systematic sampling

procedures should be planned ahead by the study sites. During the period of systematic selection, the study sites will make sure to document the sampling fraction.

- *Study site foreseeing budget limits to detail a systematic sampling procedure*

Data collected

- Hospital ward defined as "first ward of referral"

The variable "hospital wad" was interpreted differently across study sites, making comparison impossible. We further defined its meaning.

- Date of hospital discharge not required anymore in 2016-17 protocol

This variable was collected to define different level of influenza severity. As it is not part of the main study objective and it is often difficult to collect (requires following up patients), we decided to make it optional.

- Dates of antiviral treatment

The variable "antiviral treatment" was associated with "date of treatment", which was difficult to interpret by study sites. To insure that we capture patients under antiviral treatment before swabbing, we revised the question as follows: Has the patient received an antiviral treatment within the 2 weeks before swabbing

- Definition of chronic conditions:

We changed the following wording:

Cirrhosis, chronic hepatitis → chronic liver disease

Diabetes and endocrine diseases → diabetes mellitus

Due to difficulty to interpret and discussions about the underlying conditions leading to such symptoms, nutritional deficiencies was dropped from 2016-17 protocol. Instead we decided to add anemia to the 2016-17 protocol.

Person under medical supervision for obesity → obesity

To specify conditions included in these categories of chronic diseases, lists of specific conditions and their corresponding ICD-9 and 10 codes were added to the 2016-17 protocol

- GP visits in the past three months not required anymore in 2016-17 protocol

In 2015-16, this variable was collected as a proxy for health seeking behaviour (with more GP visits, more chances to be vaccinated). While this variable is likely associated with vaccination, we believe that in EU countries, health seeking behaviour is unlikely to affect the probability of hospitalisation of a patient with a SARI. GP visits is therefore not a good candidate for confounding (and is hard to collect).

- Barthel index dropped from not required anymore in 2016-17 protocol

This index score, based on ten questions, was meant to assess the level of functional impairment of the patients at the time of symptoms onset. To avoid asking this questionnaire to too many patients, we included a "filter question" to see if the patient had any functional impairment. We realised that more than two third of the patients had no functional impairment. The added value of the Barthel score information was limited as we could not stratify further than *functional impairment Yes/No.* Consequently, and because this questionnaire was labour intensive to use, we decided to drop it.

2016–17 to 2017–18 season

Definition of immunocompromised patients:

This note was added: "Patients who are only treated with glucocorticoids and have no other immune deficiency, are considered immune suppressed when treated with: High-dose corticosteroids (≥ 20 mg/day of prednisone or equivalent for ≥2 weeks) in the last three months"

Use of statins:

The following variables were added to collect information on statins:

- statin_oct: Patient was under statin treatment in october (preceeding the start of the current influenza season)
- statin_onset: Patient was under statin treatment at the time of SARI symptoms onset
- statin_name: Name of statin product used
- statin_dose: Statin dose in atorvastatin equivalents (in mg)

Other respiratory viruses:

We invited study sites to provide information on the potential presence of non-influenza viruses among patients testing negative for influenza. The following variable was added:

- resp_virus: Does the patient test positive for any non-influenza respiratory virus?

2017–18 to 2018–19 season

Use of statins:

The following variables were updated/added to collect information on statins:

- statin: Patient was under statin treatment at any point during the season
- stat_brand: Name of statin product used
- stat_type: Type of statin product used (synthetic vs natural)
- stat_dose_mg: Statin dose in atorvastatin equivalents (in mg)
- stat_dose_fr1: Frequency of statin dose (per day vs per week)
- stat_dose_fr2: Number of times statin dose given per day or week
- stat_onsetd: Date patient started statin treatment
- stat_onsety: Year; if patient started statins before this season or precise date (stat_onsetd) is NK
- stat_seas: Patient was on statin on 01-oct-2018
- stat_presymp: Patient started statin before symptom onset
- stat_prevacc: Patient started statin before vaccination

2018–19 to 2019–20 season

Updated exclusion criteria:

The following entry in the list of exclusion criteria on page 10 has been changed.

- was hospitalised < 48 hours prior to SARI onset

now reads:

- has a history of hospitalisation within the 48 hours immediately prior to this admission