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I-MOVE+

Protocol for register-based studies to measure seasonal influenza vaccine effectiveness against laboratory confirmed influenza in hospitalised patients, including ICU admitted patients, among the elderly

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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
ICU	Intensive Care Unit
ILI	Influenza-like illness
I-MOVE	Influenza Monitoring Vaccine Effectiveness
IVE	Influenza vaccine effectiveness
LCI	Laboratory Confirmed influenza
MS	Member States
OR	Odds ratio
RT- PCR	Real Time Polymerase Chain Reaction
SARI	Severe Acute Respiratory Infection
VC	Vaccination coverage
VE	Vaccine effectiveness
➢	*(Tick/check mark indicates the sections that Member States should adapt and detail in their study annexes)*

1 Background

In 2009, the European Council of Ministers recommended that all EU MS reach an influenza vaccination coverage of 75% in all risk groups by the winter season 2014-15. Risk groups are defined as individuals 60 or 65 years and older, and people with a range of underlying medical 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 revaccination is recommended. Available seasonal influenza vaccines are only moderately effective and observed VE varies from year to year, between population subgroups (age-groups, risk groups) and differs for the various influenza type and subtype outcomes measured. Variations in VE may also depends on the study-designs used.

I-MOVE (Influenza Monitoring Vaccine Effectiveness in Europe), the first network to monitor influenza vaccine effectiveness within and across the seasons in the EU and the European Economic Area (EEA), was established in 2007. The network was funded by the European Centre for Disease Prevention and Control (ECDC) and MS. It is coordinated by EpiConcept (a Small and Medium Enterprise) and includes public health institutes from the EU and EEA. In 2010, EpiConcept initiated the InNHOVE (Influenza Network of Hospitals for Vaccine Effectiveness) project, aiming at measuring effectiveness of the vaccines 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[2,3].

Building on these networks, the I-MOVE+ project targets only the elderly, as they are one of the main target groups for influenza vaccine across Europe. They also provide unique features in relation to burden of disease and immunosenescence. I-MOVE+ provide early and final influenza VE estimates in the elderly population to the WHO to complement the virological information used to select the strains included in the vaccines.

Additionally, the I-MOVE+ platform will have a particular focus on the available data and the added value of the electronic databases. Health care registries and electronic databases constitute an important data source for the estimation of effectiveness and impact of vaccines. Data are collected for other purposes

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

2 Rondy M., Puig-Barbera J., Launay O. , Duval X., Castilla J., Guevara M., Costanzo S., Campana L., K., Moren A. 2011-12 seasonal influenza vaccines effectiveness against confirmed A(H3N2) influenza hospitalisation: Pooled analysis from a European network of hospitals. A pilot study. PloS One 8(4): e59681. 2013

3 Rondy M., Launay O. , Puig-Barbera J., Gefenaite G., Castilla J., de Gaetano Donati K., Galtier F., Hak E., Guevara M., Costanzo S., European hospital IVE network, Moren A. 2012-13 Influenza vaccine effectiveness against A(H1N1)pdm09, A(H3N2) and B hospitalised influenza: estimates from a European network of hospitals. Eurosurveillance. 2015 Jan 15;20(2)

than vaccine research and therefore studies are usually less costly than studies that are based on actively collected data. In many situations, data from registries are timely and high-powered in statistical terms and are therefore useful for early reporting, e.g. mid-season estimates of influenza vaccine effectiveness. Moreover, a key advantage of electronic databases is that they may include data on past/or repeated vaccinations as well as other outcomes than the acute infection (e.g. information about hospital admissions or deaths). This allows addressing important issues such as the impact of repeat immunisation. There are also limitations in registry-based studies. Some databases contain limited information on confounding factors and little background information for laboratory data, e.g. criteria for collecting a specimen for laboratory testing.

This protocol has a particular focus on register-based influenza VE studies using available data in the electronic databases. A detailed questionnaire (see D4.1) was developed and sent to every partner in WP4 to collect information about the content of available databases. The questionnaire was based on protocols from previous studies[4][5][6][7][8][9][10] from ECDC, I-MOVE and SpID-Net. The databases include information from various sources such as GP medical records, hospital medical records, hospital discharge registers, vaccination register, laboratory databases, register of notifiable diseases and administrative population registers.

Two different study designs are presented in this generic protocol: a cohort study with calculation of person-time, and a case control study based on the test negative design, which is the main design used in influenza VE studies and is recommended by EMA[11,12,13].

This publication presents the core European protocol for register-based influenza VE-studies against laboratory confirmed influenza in hospitalised patients, including Intensive Care Unit (ICU) admitted patients, among the elderly. The protocol is based on the generic protocol for register-based studies to

[4] ECDC. Protocol for Case-Control Studies to Measure Influenza Vaccine Effectiveness in the European Union and European Economic Area Member States.

[5] ECDC. Protocol for Cohort Database Studies to Measure Influenza Vaccine Effectiveness in the European Union and European Economic Area Member States.

[6] ECDC. Surveillance of Invasive Bacterial Diseases in Europe 2012: Invasive Pneumococcal Disease, Invasive Haemophilus Influenzae Disease and Invasive Meningococcal Disease.pdf.

[7] ECDC. Surveillance of Invasive Pneumococcal Disease in Europe 2010.pdf.

[8] Valenciano et al., "First Steps in the Design of a System to Monitor Vaccine Effectiveness during Seasonal and Pandemic Influenza in EU/EEA Member States."

[9] Document prepared by Camelia Savulescu, Germaine Hanquet, Marta Valenciano, Alain Moren on behalf of SpID-net project team, "SpID-Net Generic Protocol. Active Surveillance for Invasive Pneumococcal Disease at the EU/EEA Level. January 2013."

[10] SSI. Anbefalinger for Pneumokokvaccination Udenfor Børnevaccinationsprogrammet I Danmark - Pneumokokvaccination Uden for Brnevaccinationsprogrammet I Danmark v13.ashx.

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

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

[13] European Medicines Agency. Guideline on Influenza Vaccines. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/07/WC500170300.pdf [accessed May 2015]

measure seasonal influenza vaccine effectiveness (IVE) against laboratory confirmed influenza (LCI) in hospitalised patients, including ICU admitted patients, among the elderly, developed in the previous season (2015-16) within the I-MOVE+ framework (D.4.2). It includes a plan for the pooled analysis. Site-specific protocols will be included in an annex to the generic protocol.

2 Objectives

2.1 Primary objective

The primary objective will be to measure the current seasonal influenza vaccine effectiveness against influenza laboratory confirmed hospitalisation and/or influenza laboratory confirmed ICU admission among elderly (aged ≥65 years) using health care databases and different study designs.

2.2 Secondary objective

The secondary objective will be to estimate the effectiveness of seasonal influenza vaccination among those vaccinated in current and previous three seasons compared with those who were not vaccinated during the same period.

3 Methods

3.1 Study design

Two study designs will be used to explore the primary and secondary objectives:

1) A test-negative case-control design (TNCC),

2) A cohort design.

All two studies will be performed both at country specific level and countries will provide data for pooled analyses.

3.2 Study population

3.2.1 The test-negative case-control study

- The study population consists of all individuals who in the beginning of week 40 in the current season are 65 years and above have a respiratory sample tested for influenza in the laboratory and
- are hospitalised and/or
- are hospitalised at an ICU

3.2.2 The cohort study

The study population consists of the entire elderly population in the database who in the beginning of week 40 in the current year are 65 years and above.

➢ *Each study site specifies the study population for the two designs*

3.3 Study period

The study will begin in week 40 of the current year and end in week 20 of the following year. The follow up will start in the week where the influenza virus starts circulating, and end when influenza stops circulating. It is up to each country/region to decide the influenza circulating period.

➢ *Each study site defines the study period, the period of follow-up and the methods to define the start/end of the influenza circulating period.*

3.4 Outcome

The outcome of interest will be laboratory-confirmed influenza (LCI) in patients hospitalised and laboratory-confirmed influenza in ICU- admitted patients. Studies of IVE against LCI in ICU admitted patients will be conducted if there is a sufficient number of cases for it.

More specifically, the outcomes will be:

- Laboratory confirmed influenza A overall and subtype-specific laboratory-confirmed influenza A
- Laboratory-confirmed influenza B overall and if available by lineage (B Victoria/B Yamagata)

➢ *Each study site specifies which outcomes are used*

3.5 Case/event definition

➢ *Each study site to describe how patients are selected for swabbing (criteria to select them, proportion, etc.)*

3.5.1 Test-negative case-control design

Hospitalised patient with LCI (case)

A hospitalised influenza patient will be defined as a patient hospitalised for at least 24 hours or a patient who died within 24 hours of hospitalisation with a respiratory sample positive for influenza either during the hospitalisation or within four days before the hospital admission.

Hospitalised patient negative for influenza (control)

A hospitalised control will be defined as a patient hospitalised for at least 24 hours or a patient who died within 24 hours of hospitalisation with a respiratory sample negative for any influenza both during the hospitalisation, and within four days before the hospital admission.

ICU admitted patient with LCI (case)

An ICU influenza case will be defined as a patient admitted to an ICU for at least 24 hours or a patient who died within 24 hours of hospitalisation with a respiratory sample positive for influenza either during the ICU admission, or within four days before the ICU admission.

An ICU admitted patient negative for influenza (control)

An ICU control will be defined as a patient admitted to an ICU for at least 24 hours or a patient who died within 24 hours of hospitalisation with a respiratory sample negative for any influenza both during the ICU admission, and within the four days before the ICU admission.

3.5.2 The cohort method (event)

The event in the cohort study is laboratory confirmed influenza in a hospitalised patient where the respiratory sample is positive for influenza either during the hospitalisation or within four days before the hospital admission.

Person-time is calculated from the week where the first case of laboratory confirmed influenza occurs in the elderly within the study period until the patient is hospitalised for at least 24 hours with laboratory confirmed influenza (event), or the patient dies within 24 hours after hospitalisation having laboratory confirmed influenza (event), or until date of laboratory confirmed influenza outside hospital, death or until the first week where there is no laboratory confirmed influenza case in the elderly within the study period, whatever comes first.

In both study designs (cohort and TNCC), ICU patients are a subgroup of the hospitalised patients.

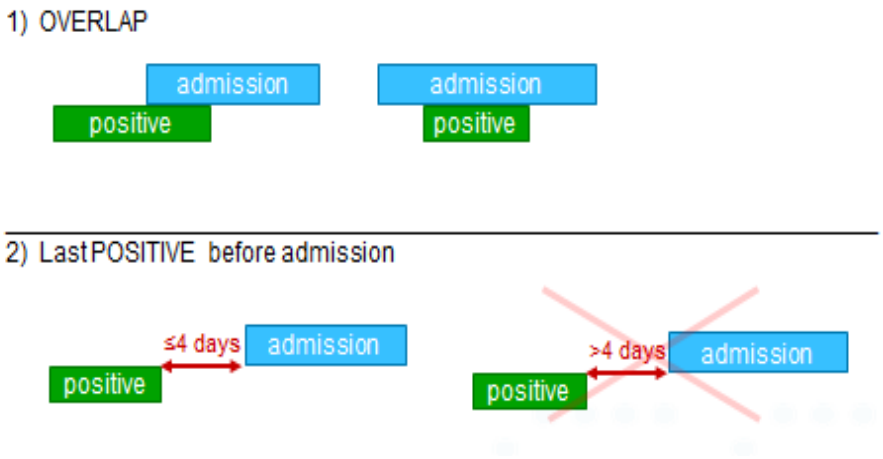
3.5.3 Exclusion criteria for the two study designs

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

- Is less than 65 years at the beginning of week 40 in the current year
- Has a hospitalisation of less than 24 hours (unless the patient dies before 24 hours)

- *Study sites specify if they use other case/event and control definitions than specified in this protocol*
- *Study sites specify to which studies they will provide data*

Figure 1: Timing of laboratory confirmation for inclusion in the study



3.6 Laboratory testing

Influenza laboratory confirmation is defined by a positive test using RT-PCR, multiplex RT-PCR and/or culture.

- *Each study site describe the test used and if there are certain procedures for collection of specimens for influenza testing, including procedures for subtyping*

3.7 Exposure (vaccination)

3.7.1 Definition of vaccination status for all three designs

The seasonal influenza vaccination will be a time dependent exposure variable with three levels:

- An individual will be considered as unvaccinated if he/she did not receive the influenza vaccine in the current season.
- An individual will be considered as intermediate if he/she did receive the influenza vaccine 0-14 before date of swabbing or date of onset if available in the current season.
- An individual will be considered as vaccinated if he/she did receive the influenza vaccine >14 days before date of swabbing or date of onset if available in the current season

- *Each study site specifies whether they will use date of onset or date of swabbing*

3.7.2 Source of vaccination status

The exposure of interest in this study will be vaccination with any influenza vaccine in the season under investigation. The vaccination history includes date of administration and type of vaccine.

The sources of information for the vaccination status may include:

- vaccination registry
- electronic patient record
- data from the patient's insurance company showing re-imbursement of influenza vaccine during the current influenza season.

➢ *Each study sites to describe how vaccination status is collected/validated*

3.8 Confounding factors and effect modifiers

3.8.1 Chronic diseases

List of underlying conditions, which could be potential confounding factor/effect modifiers:

- cirrhosis, chronic hepatitis diseases
- diabetes and endocrine
- heart disease
- hematologic cancer
- immunodeficiency and organ transplant
- lung disease
- nonhematologic cancer
- nutritional deficiencies
- renal disease
- dementia, stroke
- rheumatologic diseases
- person under medical supervision for obesity
- Anaemia

ICD codes or ICPC-2 codes are used to identify chronic diseases (see Table 1). For correspondence between different ICD versions, see annex 5. Only chronic diseases diagnosed within 5 years prior to week 40 in the current season will be included.

The variable chronic diseases will have two levels:

- An individual is considered to have no chronic diseases if none of the diseases listed in Table 1 has been diagnosed within the last 5 years
- An Individual is considered to have chronic diseases if at least one of the diseases listed in Table 1 has been diagnosed within the last 5 years

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

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

- *Each study site to define the list of chronic diseases to be included, describe the source of information and if chronic diseases 5 years back in time are included*

3.8.2 Severity/ Health seeking behaviour

The number of hospitalisations in the previous 12 months may reflect both the severity of an underlying condition and the health seeking behaviour, and both can be confounding variables.

The variable severity/ health seeking behaviour will be coded with three levels

- Individuals who have no hospitalisations in the previous 12 months
- Individuals who have 1-5 hospitalisations in the previous 12 months
- Individuals who have >5 hospitalisations in the previous 12 months

3.8.3 Previous influenza vaccination

Vaccination against influenza in the previous three seasons will be collected.

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

- vaccination registry
- electronic patient record
- data from the patient's insurance company showing re-imbursement of influenza vaccine during the current influenza season.

The variable "previous influenza vaccination" has eight possible combinations. Vaccination status three years ago is listed first, followed by the vaccination status two years ago and last years vaccination status listed last (2013-14, 2014-15, 2015-16) :

- y,y,y
- y,y,n
- y,n,y
- n,y,y
- n,n,y
- n,y,n
- y,n,n
- n,n,n

3.9 Sample size

This WP use data from existing databases collected anyway as part of the established influenza surveillance, therefore no sample size calculations are made. If the power of the studies in the first season is limited, it should be examined if it is possible to increase the number of samples taken at hospitals.

3.10 Data

3.10.1 Collected information

Collected information in each study site (see also Annex 1: List of variables, definition and coding):

- study identification
- 5-year age categories (65-69, 70-74, 75-79, 80-84, >84),
- gender
- date of hospital admission and date of hospital discharge / length of hospital stay
- date of swabbing
- laboratory results type, subtype and lineage
- underlying chronic conditions (Y/N)
- number of hospitalisations for the chronic diseases in the previous 12 months
- current season influenza vaccination including date and type
- influenza vaccination in the three previous seasons

➢ *Each study site to specify the data collected*

3.10.2 Data validation

Each data custodian is responsible for the validity of the databases.

➢ *Each study site to specify how data are validated and potential limitations with the data*

3.11 Data management

3.11.1 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. 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.

➢ *Study teams to specify the data checking and cleaning process*
➢ *Each study site to describe the degree of evaluation of missing information.*

3.11.2 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. The study sites will send aggregated data to the coordinating team based on predefined aggregation levels (See Annex 2-3). A country identifier will be included in each aggregation level.

3.12 Data Analysis

For the two study designs, the analysis will be carried out at country level and as a pooled analysis.

3.12.1 Descriptive analysis

Study population will be described by baseline characteristics. Baseline characteristics of cases/events and controls will be compared using the chi-square test and t-test. The association between vaccination status and baseline characteristics will be measured for both the case group and the control groups.

Each study site will prepare and send to the coordinating team (SSI) the following pre-designed table (table 2) with the descriptive characteristics of their study population for the two study designs.

Table 2. Baseline characteristics of the population under study in secondary care, cohort design and/or TNCC

Baseline characteristics	Country/region					
	Vaccination status 2016/17					
	No			Yes		
	N	(%) row	% whole pop.	N	(%) row	(%) whole pop.
Age						
65-69						
70-74						
75-79						
80-84						
85+						
Gender						
Female						
Male						
N. of hospitalisations (for chronic conditions)						
0						
0-5						
>5						
Previous vaccinations						
2013-14						
2014-15						
2015-16						

Furthermore, each study site will send to the coordinating team (SSI) the data needed to elaborate an epidemiological curve of the influenza season by week and it should be subtype specific.

3.12.2 Analysis of primary objective

Measure of effect

OR is calculated in the TND study. IRR is calculated in the cohort study. Vaccine effectiveness will be calculated as respectively VE= 1 – OR and VE= (1-IRR) and expressed as percentage and with 95% confidence limits.

The test-negative case-control study

Odds Ratios (OR) will be calculated using the Mantel-Haenszel method with stratification on the three previous years of vaccination, age in five years intervals, gender, underlying illness and hospitalisations for chronic diseases the previous 12 months. Vaccine effectiveness (VE) will be calculated as 1-OR. VE will be estimated for influenza A overall, influenza B overall and by subtypes and linages separately if possible.

Effect modification will be assessed by 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. A factor will be considered as a confounder if it changes the pooled overall estimate by more than 10%.

The cohort study

Seasonal influenza vaccination is a time dependent exposure variable with three levels "Not vaccinated", "intermediate" and "fully vaccinated". The baseline risk for influenza will be stratified on country. The analysis will be conducted using the following aggregation level: the previous three years of vaccination, age in five years intervals, gender, underlying illness, number of hospitalisations in the previous 12 months and month/week in season. Within each aggregation level, events and person years are counted by seasonal vaccination status in current year.

Estimates of the effect of current season's vaccination will be adjusted for confounding factors.

Country specific estimates of the incidence rate ratios (IRR) will be calculated with Poisson regression. The pooled estimates will be calculated using Poisson regression.

3.12.3 Analyses of secondary objectives (Stratified analysis)

The effect of several years of seasonal influenza vaccination is analysed and VE among those vaccinated in all previous three seasons and current season is compared with those who were not vaccinated in any of these seasons. Differences between these two groups other than vaccination status is adjusted by propensity score.

3.12.4 Propensity score methodology

The propensity score methodology will be used to adjust for confounding factors.

The propensity score calculation should be flexible and reflect and reflect the confounders relevant for the country-specific data. The inclusion of covariates for health seeking behaviour or less severe chronic diseases can be considered.

Proposal for propensity score calculation:

1. Consider a list of diseases (heart, liver, diabetes, endocrine, hemotogic, HIV, kidney, cancer, lung…). We considered about 30 diseases in the list.
2. For each person in the entire cohort (all country), create a vector of binary variables indicating whether the person had had the disease within the last 5 years from 1 October 2016.
3. Identify also the variables:
    - $\text{Vaccination}_{2016}$ (yes/no), $\text{vaccination}_{2015}$, $\text{vaccination}_{2014}$, $\text{vaccination}_{2013}$
    - Age (5 years interval) and gender
    - And any other variable relevant for the specific study site
4. For each combination of gender, age, $\text{vaccination}_{2014}$, $\text{vaccination}_{2013}$, $\text{vaccination}_{2012}$, etc.,

create a logistic regression model with $vaccination_{2016}$ as outcome and the diseases as predictors:

$$\textbf{Probability (vaccination}_{2015}\textbf{= ”yes”) = 1/((1+exp(-(b}_1\textbf{heart + b}_2\textbf{liver+ b}_3\textbf{diabetes +…)))}$$

No interaction between the diseases to avoid making it too complicated.

5. For each person in the entire cohort, calculate the chance of vaccination. Persons with probability of same order should be grouped together, with in total 5 groups (1-5). The 5 groups are made to be of same size approximately.

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 Table 3 will be generated for each study site. Useful information includes numbers of cases and controls (overall and vaccinated) and presentation of results for different stratifications.

Table 3. Output tables

Denmark																		
Previously vaccines			Un-vaccinated			Vaccinated				Absolute risk		Mantel Haenzel statistics (OR)						
2011/2012	2012/2013	2013/2014	events	non-events	Total	events	non-events	Total	Total	Vaccinated	nvaccinated	OR_j	R_j	S_j	tæller1	nævner1	tæller2	nævner2
no	no	no	276	927584	927860	27	78027	78054	1.005.914	0,03%	0,03%	1,16	24,89752	21,40884	22,959435	24,897524	21,680408	21,40884
yes	no	no	12	32370	32382	5	14213	14218	46.600	0,04%	0,04%	0,95	3,473176	3,66	2,4129629	3,473176	3,6029706	3,66
no	yes	no	9	18705	18714	3	13265	13268	31.982	0,02%	0,05%	0,47	1,754581	3,732881	1,0263491	1,7545807	2,9117954	3,732881
yes	yes	no	13	24517	24530	21	39017	39038	63.568	0,05%	0,05%	1,02	8,099311	7,9791876	3,1264298	8,099311	8,0529419	7,9791876
no	no	yes	12	45114	45126	24	83020	83044	128.170	0,03%	0,03%	1,09	8,447655	7,7728017	2,9750353	8,4476555	8,2099903	7,7728017
yes	no	yes	9	16049	16058	18	47016	47034	63.092	0,04%	0,06%	0,68	4,578742	6,7067774	1,1660218	4,5787422	5,1206673	6,7067774
no	yes	yes	5	20247	20252	25	77981	78006	98.258	0,03%	0,02%	1,30	5,151489	3,9681756	1,0628242	5,1514889	4,9073548	3,9681756
yes	yes	yes	42	72868	72910	239	580759	580998	653.908	0,04%	0,06%	0,71	26,63288	37,301697	2,9775592	26,632878	27,825653	37,301697
Total			**378**	########	1157832	**362**	**933298**	933660	2.091.492	**0,04%**	**0,03%**	**1,19**	0,00541	**Raw OR=1,19**	37,71	83,04	82,31	92,53
												0,8974	0,011125	**MH OR=0,90(0,73-1,11)**				

3.12.5 Pooled analysis (see annex 4)

OR's will be pooled using the Mantel Haenzel method by applying stratification on country and the selected confounders. The Breslow-Day-Tarone will be used to test whether we can assume heterogeneity of the OR's. The power of this heterogeneity test might be low because some strata's have few observations.

IRR will be calculated using a Poisson regression model. The estimated IRR will be adjusted for previous influenza vaccines and for country as a random intercept. All parameters except a common IRR will be estimated separately for each country. Score-tests (generalization of Log-rank tests) will be used to test heterogeneity of IRR's across countries.

3.12.6 Statistical software

All statistical analyses for the pooled analysis will be carried out using SAS version 9.4 (Carry, NC)

3.13 Potential biases

3.13.1 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 VE. If negative confounding is present, the VE will be underestimated or even be negative. Adjustment for potential negative confounding factors documented in the study (e.g. presence of chronic diseases) will minimise negative confounding.

3.13.2 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 analysis with adjustment for confounders such as previous vaccinations and comorbidity.

3.13.3 Information bias

As there is no data on onset of symptoms, it is not possible to exclude patients for whom the time between onset of symptoms and sampling is larger than required to detect the influenza virus.

Additionally, the collection of clinical samples is based on the clinician´s judgement, meaning that the collection might not be random in relation to the exposure, comorbidities and other confounders or effect modifiers.

3.13.4 Unmeasured confounding

Studies based on databases are limited by the available information. Known confounders like smoking, functional status, etc. are often not available in the databases and can therefore not be adjusted for in the analysis.

3.13.5 Misclassification of exposure

There might be an underreporting of vaccines administered. However, as the vaccination information is collected before the outcome occurs there is no reason to expect that the underreporting should be different among those who are diagnosed with influenza compared to those who are not diagnosed with influenza, which means that the underreporting is a non-differential misclassification which tends to weaken estimates. Only if swabbing is dependent on vaccination status differential misclassification could occur, but as the outcomes to be studied are severe outcomes of influenza we assume that the threshold for swabbing is likely to be independent of vaccination status.

- *Each study site to describe the completeness of the vaccination registers*

3.13.6 Pooled estimate and its bias

Any bias in the country specific studies influences the pooled estimate. The power of the test for the presence of heterogeneity between individual studies will be low when the sample size per level of confounding 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.

3.14 Consent

Each study will comply with national ethics committee requirements.

- *Each study site to describe the procedures to comply to the national ethics committee requirements*
- *Each study site to send a copy of the ethical approval to the coordinating centre*

3.15 Dissemination of results

Final estimates will be disseminated at the end of the season to the members of the consortium including the Commission Services.

3.15.1 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. If an article is published on the pooled results, the actual authorship will be discussed and agreed with the study teams at the beginning of the study.

4 Logistical aspects

4.1 Study leader

In each study site, a principal investigator will lead the study and act as contact person for the pooled study. Statens Serum Institut is in charge of the pooled analysis.

4.2 Teleconferences

Teleconferences will be arranged for participating study sites along with the technical meetings. During the season, at least a monthly teleconference will be arranged where each study site can update each other on how the influenza season is developing and where difficulties and uncertainties can be discussed.

4.3 Computer support

Data collection and entry will be conducted at study site level. Statens Serum Institute will provide a structured summary statistics excel sheet to illustrate how data should be aggregated within each country. It is up to the study site in which format they decide to provide data.

4.4 Report

Statens Serum Institut will write a final report with both country specific VE estimates as well as the pooled VE analysis, which will be sent to EpiConcept and the participating study sites.

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6 Annexes

Annex 1: List of variables, definitions and coding needed before aggregation; IMOVE+ hospital register-based IVE studies

	Variable	Type	Values and coding	Definition
General	Country	Categorical	Coded according to international country codes	Identifier uniquely identifying the country
	Age	Numeric (categorical)	1 = 65-69	Age in 5 years categories
			2= 70-74	
			3= 75-79	
			4= 80-84	
			5= >84	
	Sex	Categorical (binary)	0 = female	Sex of study participant
			1 = male	
	Variable	**Type**	**Values and coding**	**Definition**
Laboratory confirmation	Lab_result	Numeric (categorical)	0 = Negative	Laboratory result (influenza positive or negative)
			1 = Positive	
	Virus_type	Text	A= influenza A	Laboratory result: virus type
			B= influenza B	
	Virus subtype	Text	AH1N1= influenza H1N1	Laboratory result: virus subtype
			AH3N2= influenza H3N2	
			VIC= influenza B Victoria	Laboratory result: influenza B lineage
			YAM= influenza B Yamagata	
	Date_swab	Dd/mm/yy		Date of swabbing/ date of onset
	Date_time_admis			Date and time of admission to hospital
	Date_time_discharge			Date and time of discharge from hospital
Vaccination status	VAC_current	Numeric (categorical)	0 = No	Vaccination status. In test-negative case-control study and the screening study 0= not-vaccinated 1=vaccinated In the cohort study 0= not-vaccinated 1=vaccinated and 2=intermedialte
			1 = Yes	
			2 = Intermediate	
	Vac_type	Text		Type of vaccine administered
	Vac:date	Dd/mm/yy		Date of vaccination
	Vac_n_1	Numeric (categorical)	0 = No	Received seasonal influenza vaccination in previous season (2014-2015)
			1 = Yes	
	Vac_n_2	Numeric (categorical)	0 = No	Received seasonal influenza vaccination in season (2013-2014)
			1 = Yes	
	Vac_n_3	Numeric (categorical)	0 = No	Received seasonal influenza vaccination in season (2012-2013)
			1 = Yes	

	Variable	Type	Values and coding	Definition
	Chron_disease	Numeric (categorical)	0=none 1=at least one	Presence of at least one chronic disease or absence of any chronic disease
Underlying chronic conditions	Cirrhosis	Numeric (categorical)	0 = No 1 = Yes	Cirrhosis
	Diabetes	Numeric (categorical)	0 = No 1 = Yes	Diabetes and endocrine
	heart_dis	Numeric (categorical)	0 = No 1 = Yes	Heart disease
	hema_cancer	Numeric (categorical)	0 = No 1 = Yes	Hematologic cancer
	Immuno	Numeric (categorical)	0 = No 1 = Yes	Immunodeficiency and organ transplant
	Lungdis	Numeric (categorical)	0 = No 1 = Yes	Lung disease
	nonhem_cancer	Numeric (categorical)	0 = No 1 = Yes	Nonhematologic cancer
	nut_def	Numeric (categorical)	0 = No 1 = Yes	Nutritional deficiencies
	ren_disease	Numeric (categorical)	0 = No 1 = Yes	Renal disease
	dem_stroke	Numeric (categorical)	0 = No 1 = Yes	Dementia, stroke
	Rheumat	Numeric (categorical)	0 = No 1 = Yes	Rheumatologic diseases
	Obese	Numeric (categorical)	0 = No 1 = Yes	Person is under medical supervision for obesity.
	Sev_chron	Numeric (categorical)	0= no hospitalisations 1=1-5 hospitalisations 2=>5 hospitalisations	Number of hospitalisations previous 12 months for the chronic disease,

Annex 2: Template for pooling data for cohort study using person time

Previous vaccination (2012-13, 2013-14, 2014-15)	Age categories	Gender	No. Hospitalisations previous 12 months	Chronic disease	Vaccination status	Time in season (month)	Propensity score	Influenza A overall (both subtyped and not subtyped)	
								events	Person years
yes, yes, yes	65-69	M	0	0	0	Oct	1		
yes, yes, yes	65-69	M	0	0	1	Oct	1		
yes, yes, yes	65-69	M	0	0	2	Oct	1		
yes, yes, yes	65-69	M	0	0	0	Oct	2		
yes, yes, yes	65-69	M	0	0	1	Oct	2		
yes, yes, yes	65-69	M	0	0	2	Oct	2		
yes, yes, yes	65-69	M	0	0	0	Oct	3		
yes, yes, yes	65-69	M	0	0	1	Oct	3		
yes, yes, yes	65-69	M	0	0	2	Oct	3		
yes, yes, yes	65-69	M	0	0	0	Oct	4		
yes, yes, yes	65-69	M	0	0	1	Oct	4		
yes, yes, yes	65-69	M	0	0	2	Oct	4		
yes, yes, yes	65-69	M	0	0	0	Oct	5		
yes, yes, yes	65-69	M	0	0	1	Oct	5		
yes, yes, yes	65-69	M	0	0	2	Oct	5		
yes, yes, yes	65-69	M	0	0	0	Nov	1		
yes, yes, yes	65-69	M	0	0	1	Nov	1		
yes, yes, yes	65-69	M	0	0	2	Nov	1		
yes, yes, yes	65-69	M	0	0	0	Nov	2		
yes, yes, yes	65-69	M	0	0	1	Nov	2		
yes, yes, yes	65-69	M	0	0	2	Nov	2		
yes, yes, yes	65-69	M	0	0	0	---	---		
yes, yes, yes	65-69	M	0	0	1	---	---		
yes, yes, yes	65-69	M	0	0	2	---	---		
yes, yes, yes	65-69	M	0	1	0	Oct	1		
yes, yes, yes	65-69	M	0	1	1	Oct	1		
yes, yes, yes	65-69	M	0	1	2	Oct	1		
yes, yes, yes	65-69	M	0	1	0	Oct	2		
yes, yes, yes	65-69	M	0	1	1	---	---		
yes, yes, yes	65-69	M	0	1	2	---	---		
yes, yes, yes	65-69	M	1-5	0	0	Oct	1		
yes, yes, yes	65-69	M	1-5	0	1	Oct	1		
yes, yes, yes	65-69	M	1-5	0	2	Oct	1		
yes, yes, yes	65-69	M	1-5	0	0	Nov	2		
yes, yes, yes	65-69	M	1-5	0	1	---	---		
yes, yes, yes	65-69	M	1-5	0	2	---	---		
yes, yes, yes	65-69	M	>5	0	0	Oct	1		
yes, yes, yes	65-69	M	>5	0	1	Oct	1		
yes, yes, yes	65-69	M	>5	0	2	Oct	1		
yes, yes, yes	65-69	M	>5	0	0	Nov	2		
yes, yes, yes	65-69	M	>5	0	1	---	---		
yes, yes, yes	65-69	M	>5	0	2	---	---		
yes, yes, yes	65-69	F	0	0	0	Oct	1		
yes, yes, yes	65-69	F	0	0	1	Oct	1		
yes, yes, yes	65-69	F	0	0	2	Oct	1		
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- The propensity score calculation should be flexible and reflect confounders relevant for the country-specific data. This variable will have 5 levels (1,2,3,4,5)
- In the example above only influenza A overall are shown. In the complete aggregation table, that each study site will receive also influenza A H3N2, influenza A H1N1, influenza B overall, influenza B-Victoria and influenza B-Yamagata are included.
- The same output as above should be generated for the other age categories 70-74, 75-79, 80-84, >84 with previous vaccinations Yes, Yes, yes.

- The same output as above should be generated for the various combinations of previous vaccinations no,no,no and yes,no,no and yes,yes,no and no,yes,yes and no,yes,no and no,no,yes and yes,no,yes for each of the age categories 65-69, 70-74, 75-79, 80-84, >84

Annex 3: Template for pooling data for the test negative case control design (TNCC)

Previous vaccination (2012-13, 2013-14, 2014-15)	Age categories	Gender	No. Hospitalisations previous 12 months	Chronic disease	Vaccination status	Time (month)	Propensity score	**Influenza A overall (both subtyped and not subtyped)**	
								Test Negative Case Control Design (TNCC)	
								Cases	**Controls**
yes, yes, yes	65-69	M	0	0	0	Oct	1		
yes, yes, yes	65-69	M	0	0	1	Oct	1		
yes, yes, yes	65-69	M	0	0	2	Oct	1		
yes, yes, yes	65-69	M	0	0	0	Oct	2		
yes, yes, yes	65-69	M	0	0	1	Oct	2		
yes, yes, yes	65-69	M	0	0	2	Oct	2		
yes, yes, yes	65-69	M	0	0	0	Oct	3		
yes, yes, yes	65-69	M	0	0	1	Oct	3		
yes, yes, yes	65-69	M	0	0	2	Oct	3		
yes, yes, yes	65-69	M	0	0	0	Oct	4		
yes, yes, yes	65-69	M	0	0	1	Oct	4		
yes, yes, yes	65-69	M	0	0	2	Oct	4		
yes, yes, yes	65-69	M	0	0	0	Oct	5		
yes, yes, yes	65-69	M	0	0	1	Oct	5		
yes, yes, yes	65-69	M	0	0	2	Oct	5		
yes, yes, yes	65-69	M	0	0	0	Nov	1		
yes, yes, yes	65-69	M	0	0	1	Nov	1		
yes, yes, yes	65-69	M	0	0	2	Nov	1		
yes, yes, yes	65-69	M	0	0	0	Nov	2		
yes, yes, yes	65-69	M	0	0	1	Nov	2		
yes, yes, yes	65-69	M	0	0	2	Nov	2		
---	---	---	---	---	---	---	---		
---	---	---	---	---	---	---	---		
---	---	---	---	---	---	---	---		
yes, yes, yes	65-69	M	0	1	0	Oct	1		
yes, yes, yes	65-69	M	0	1	1	Oct	1		
yes, yes, yes	65-69	M	0	1	2	Oct	1		
yes, yes, yes	65-69	M	0	1	0	Oct	2		
---	---	---	---	---	---	---	---		
---	---	---	---	---	---	---	---		
yes, yes, yes	65-69	M	1-5	0	0	Oct	1		
yes, yes, yes	65-69	M	1-5	0	1	Oct	1		
yes, yes, yes	65-69	M	1-5	0	2	Oct	1		
yes, yes, yes	65-69	M	1-5	0	0	Nov	2		
---	---	---	---	---	---	---	---		
---	---	---	---	---	---	---	---		
yes, yes, yes	65-69	M	>5	0	0	Oct	1		
yes, yes, yes	65-69	M	>5	0	1	Oct	1		
yes, yes, yes	65-69	M	>5	0	2	Oct	1		
yes, yes, yes	65-69	M	>5	0	0	Nov	2		
---	---	---	---	---	---	---	---		
---	---	---	---	---	---	---	---		
yes, yes, yes	65-69	F	0	0	0	Oct	1		
yes, yes, yes	65-69	F	0	0	1	Oct	1		
yes, yes, yes	65-69	F	0	0	2	Oct	1		
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- The propensity score calculation should be flexible and reflect confounders relevant for the country-specific data. This variable will have 5 levels (1,2,3,4,5)
- In the example above only influenza A overall are shown. In the complete aggregation table, that each study site will receive also influenza A H3N2, influenza A H1N1, influenza B overall, influenza B-Victoria and influenza B-Yamagata are included.

- The same output as above should be generated for the other age categories 70-74, 75-79, 80-84, >84 with previous vaccinations Yes, Yes, yes.
- The same output as above should be generated for the various combinations of previous vaccinations no,no,no and yes,no,no and yes,yes,no and no,yes,yes and no,yes,no and no,no,yes and yes,no,yes for each of the age categories 65-69, 70-74, 75-79, 80-84, >84

Annex 4: Examples of pooled analyse for cohort studies

Previously vaccines	current-season vaccine	month	Person-time	Events	HR
yes,yes,yes	yes	october	61336	417	0,568680906
yes,yes,yes	no	october	9703	116	
yes,yes,no	yes	october	3736	35	0,769904614
....	....	....	....	....	....
no,no,yes	no	january	5489	42	
no,no,no	yes	january	7744	48	0,831429581
no,no,no	no	january	103554	772	

IRR: 0.70(0.62- 0.80)

Calculated with Poisson

Or, data aggregated on explanatory variables and riskset (riskset is a crosssection at each time an event occur)

Previously vaccines	current-season vaccine	Riskset time	Number of persons at risk	Number of events
yes,yes,yes	Yes	31dec2014	39309	1
yes,yes,yes	no	31dec2014	472730	1
yes,yes,yes	Yes	7jan2015	39326	0
yes,yes,yes	no	7jan2015	472638	1
yes,yes,yes	Yes	14jan2015	39271	0
....	....	....	....	....
....	....	....	....	....

IRR=0.93(0.74-1.18) Calculated with Cox

Total (DK+?): IRR=0.75(0.67- 0.84)

Descriptive analysis

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

- Beginning of influenza period, peak, end
- Beginning of vaccination campaigns for seasonal vaccine
- Vaccines used
- Estimated vaccine coverage in the country/region
- Vaccine coverage by 5-year age interval, presence of chronic diseases, no of hospitalisations previous 12 month
- Number of patients swabbed distribution of negative and positive

Annex 5. Correspondence table ICD codes

(ICD - COD SL-2012)

Annex 6: Study-specific annexes

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

- Study population for each design
- Date of the start of the seasonal vaccination campaign
- Outcomes used
- How patients are selected for swabbing (criteria to select them, proportion, etc.)
- If the study site uses other case/event and control definitions than specified in this protocol
- To which studies the study site will provide data
- Description of the test used and if there are certain procedures for collection of specimens for influenza testing, including procedures for subtyping
- Whether the study site will use date of onset or date of swabbing
- How vaccination status is collected/validated
- Definition of the list of chronic diseases to be included, describe the source of information and if chronic diseases 5 years back in time are included
- Data collection
- Data validation and potential limitations with the data
- Data checking and cleaning process
- Degree of evaluation of missing information.
- Completeness of the vaccination registers
- Procedures to comply to the national ethics committee requirements
- Ethical approval

Annex 7: Ethical clearance