

**ECDC TECHNICAL REPORT**

**Core protocol for ECDC studies of vaccine effectiveness against symptomatic laboratory-confirmed influenza or SARS-CoV-2 infection at primary care level**

Version 1.0, September 2023



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## Abbreviations

COVID-19	Coronavirus disease 2019
CVE	COVID-19 vaccine effectiveness
EEA	European Economic Area
EU	European Union
GP	General Practitioner
ICD	International classification of diseases
ILI	Influenza-like illness
I-MOVE	Influenza – Monitoring Vaccine Effectiveness in Europe
IQR	Interquartile range
IVE	Influenza vaccine effectiveness
OR	Odds ratio
RT-PCR	Reverse-transcriptase polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
VC	Vaccination coverage
VE	Vaccine effectiveness

➤ *Arrow marks indicate the sections that countries/study sites should adapt and provide details for in their study annexes.*

## Executive summary

The end of 2019 saw the emergence of a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19). As of August 2023, eight vaccines had been authorised by the European Commission based on the scientific opinion of the European Medicines Agency (EMA) for use in the European Union (EU): Bimervax (previously COVID-19 Vaccine HIPRA), Comirnaty, COVID-19 Vaccine Valneva, Jcovden (previously COVID-19 Vaccine Janssen), Nuvaxovid (previously Novavax), Spikevax (previously COVID-19 Vaccine Moderna), Vaxzevria (previously COVID-19 Vaccine AstraZeneca), and VidPrevtyl Beta (indicated as a booster). In addition, four adapted vaccines have also been authorised: Comirnaty Original/Omicron BA.1, Comirnaty Original/Omicron BA.4-5, Spikevax bivalent Original/Omicron BA.1, and Spikevax bivalent Original/Omicron BA.4-5. Many other vaccines are under rolling review [4].

Influenza viruses undergo frequent genetic and antigenic changes. The influenza vaccine is reformulated each year and annual re-vaccination is recommended. Observed influenza vaccine effectiveness (IVE) varies from year to year between population sub-groups (age groups, risk groups) and differs for the various influenza types, subtypes and genetic clades, and outcomes measured. Immunological correlates of protection are not well defined. In 2017, the European Medicines Agency (EMA) formally adopted new guidelines on influenza vaccines covering, inter alia, post-authorisation studies of vaccine effectiveness, including brand-specific IVE data [5].

There is variation among the available influenza vaccine products currently in use for EU/EEA immunisation programmes, the target groups for vaccination, and the vaccination coverage across countries [6]. New vaccines are being developed for which limited or no effectiveness data are yet available in the EU. A comparison by vaccine type (adjuvanted vs nonadjuvanted, live attenuated vs inactivated, egg- vs cell-based), group (split virion, subunit, etc.) and product could provide essential information for vaccine recommendations and health economic assessments.

In 2020, the European Commission stressed the importance of continuously monitoring the safety and effectiveness of vaccines in the EU/EEA in the post-authorisation phase, with particular emphasis on COVID-19 vaccines in the context of the ongoing pandemic [7]. The 2018 'Council Recommendation on Strengthened Cooperation against Vaccine-preventable Diseases' already called on ECDC and EMA to cooperate to ensure the continued monitoring of vaccines and vaccination in use in EU/EEA vaccination programmes [8]. This request was subsequently formalised as part of the extended EMA regulatory mandate [9] and ECDC's newly amended mandate [10], requiring the two agencies to develop a structured and independent post-authorisation vaccine monitoring platform, initially prioritising COVID-19 vaccines. ECDC and EMA officially established and launched the platform in May 2022, with the intention of bringing together public health and regulatory experts to discuss the studies needed to generate real-life evidence on the safety and effectiveness of vaccines in use in EU/EEA immunisation programmes.

From 2020, ECDC began building infrastructure to perform COVID-19 vaccine effectiveness (CVE) studies using the lessons learned from other vaccine effectiveness studies. One such study was the ECDC-funded project I-MOVE (Influenza – Monitoring Vaccine Effectiveness in Europe), under which IVE was measured in Europe using primary care sentinel surveillance systems since the 2007–2008 influenza season [11, 12]. The infrastructure will be used to build a system that regularly monitors vaccine effectiveness and performs studies, including impact and burden of disease studies, in different settings. Depending on the setting, information will be provided on different outcomes (severe disease, moderate disease, transmission, etc). The overall project is called VEBIS (Vaccine Effectiveness, Burden and Impact Studies) and it includes different networks of study sites/countries/infrastructures, where the multi-country studies are conducted.

This document presents the core protocol for ECDC studies of CVE and IVE against symptomatic laboratory-confirmed influenza or SARS-CoV-2 infection, respectively, at primary care level. This core protocol presents the main elements for a multicentre (multi-country) study of IVE/CVE at primary care level, outlining the agreed methods for collecting data related to influenza and SARS-CoV-2 at country level, and includes a plan for the pooled analysis. The combination of data from multiple study sites will allow for studies with more statistical power to meet more specific objectives. The protocol can be implemented for COVID-19 and/or influenza.

The proposed method is a case-control study using a test negative design. The study population consists of people of all ages, belonging to the target group for COVID-19 or influenza vaccination, presenting to primary care with symptoms of acute respiratory infection (ARI) or influenza-like illness (ILI), and no contraindication for being vaccinated with the vaccine of interest.

This protocol is adapted from the I-MOVE and I-MOVE+ generic protocols [1, 2] and the I-MOVE-COVID-19 generic protocol [3] and is written in a generic manner. The specificities of each study site's influenza and/or COVID-19 VE study can be detailed in the individual site protocol annexes (Annex 5).

This core protocol is primarily intended to guide the implementation of ECDC-funded studies. However, ECDC encourages the conduct of vaccine effectiveness studies, using this protocol as a basis, in countries not currently planning to participate in ECDC-funded studies. The use of consistent protocols will facilitate the comparability of study results across studies, countries, and study sites.

This document presents version 1.0 of the core protocol which is an evolving document. This document will be updated and revised as necessary.

Protocols for IVE and/or CVE studies in other settings, such as in hospitals or in long-term care facilities, are also available on [ECDC's website](#).

# 1 Background

## 1.1 Context: COVID-19

The end of 2019 saw the emergence of a novel severe acute respiratory syndrome SARS-CoV-2, which can cause coronavirus disease 2019 (COVID-19).

As of August 2023, eight authorised COVID-19 vaccines and four adapted vaccines have received conditional marketing authorisation by the European Commission (EC) based on the scientific opinion of the European Medicine Agency (EMA) [4], as shown in Table 1 below.

**Table 1. COVID-19 vaccines authorised and in use in the EU/EEA as of August 2023**

Vaccine	Age of recommendation	Primary dose regimen	Booster dose
Bimervax (previously COVID-19 Vaccine HIPRA)	16 years +	No	May be given as a booster at least six months after a previous mRNA COVID-19 vaccine.
Comirnaty	Six months +	Single dose may be given in individuals aged five years + for primary vaccination regardless of prior COVID-19 vaccination status.  In children aged from six months to four years, two doses are given three weeks apart, followed by a third dose given at least eight weeks after the second dose.  Different doses are available for adults and adolescents (aged 12 years +), children aged five to 11 years, and children aged six months to four years.	May be given as a booster at least three months after the most recent dose in individuals aged six months +.  Different doses are available for adults and adolescents (aged 12 years +), children aged five to 11 years, and children aged six months to four years.
Comirnaty Original/Omicron BA.1	12 years +	No	May be given as a booster at least 3 months after the most recent dose in individuals aged 12 years +.
Comirnaty Original/Omicron BA.4-5	Six months +	Single dose may be given in individuals aged five years + for primary vaccination regardless of prior COVID-19 vaccination status.  In children aged from six months to four years, two doses are given three weeks apart, followed by a third dose given at least eight weeks after the second dose.  Different doses are available for adults and adolescents (aged 12 years +), children aged five to 11 years, and children aged six months to four years.	May be given as a booster at least three months after the most recent dose in individuals aged six months +.  Different doses are available for adults and adolescents (aged 12 years +), children aged five to 11 years, and children aged six months to four years.
COVID-19 vaccine Valneva (inactivated, adjuvanted)	18 to 50 years	Two doses, given four weeks apart.	No
Jcovden	18 years +	Single dose.	May be used as a booster dose given at least two months after the first dose of Jcovden.  A booster dose may also be given after primary vaccination with an mRNA or adenoviral vector vaccine, with the timing according to the official recommendations issued at national level by public health bodies.



Vaccine	Age of recommendation	Primary dose regimen	Booster dose
Nuvaxovid	12 years +	Two doses, given three weeks apart.	<p>May be used as a booster dose for individuals aged 18 years + around six months after primary vaccination with Nuvaxovid.</p> <p>A booster dose of Nuvaxovid may also be given after primary vaccination with an mRNA vaccine or adenoviral vector vaccine, according to the official recommendations issued at national level by public health bodies.</p>
Spikevax	Six months +	<p>Two doses, given 28 days apart.</p> <p>Different doses are available for adults and adolescents (aged 12 years +), children aged six to 11 years, and children aged six months to five years.</p> <p>An additional dose of Spikevax may be given to people aged six years + with a severely weakened immune system, at least 28 days after their second dose.</p>	<p>May be given as a booster in individuals aged six years + at least three months after the second dose of Spikevax, or another mRNA vaccine or an adenoviral vector vaccine.</p> <p>Different doses are available for adults and adolescents (aged 12 years +), and children aged six to 11 years.</p>
Spikevax bivalent Original/Omicron BA.1	Six years +	No	<p>May be given to individuals aged six years + at least three months after primary vaccination or a booster dose with a COVID-19 vaccine.</p> <p>Different doses are available for adults and adolescents (aged 12 years +), and children aged six to 11 years.</p>
Spikevax bivalent Original/Omicron BA.4-5	Six years +	No	<p>May be given to individuals aged six years + at least three months after primary vaccination or a booster dose with a COVID-19 vaccine.</p> <p>Different doses are available for adults and adolescents (aged 12 years +), and children aged six to 11 years.</p>
Vaxzevria	18 years +	Two doses, with second dose given between four and 12 weeks after the first dose.	<p>A booster dose may be given at least three months after the second dose of Vaxzevria.</p> <p>A booster dose of Vaxzevria can also be given to adults who have had two doses of an authorised mRNA COVID-19 vaccine according to the official recommendations issued at national level by public health bodies.</p>
VidPrevtyn Beta	18 years +	No	May be used as a booster dose at least four months after a previous mRNA or adenoviral vector COVID-19 vaccine.

By January 2021, all 30 EU/EEA countries had launched COVID-19 vaccination campaigns, and different COVID-19 vaccines have been gradually introduced as they have become available through the EU Vaccines Strategy [13]. Beside healthcare workers and residents in long-term care facilities, the vaccination strategy in the general population had an age-based staggered approach prioritising elderly people. Most of the vaccines used to date including booster vaccines were mRNA vaccines [14].

Post-marketing CVE studies are important to determine whether vaccines are effective or not among the target group for vaccination in real life conditions and over time. Under real life conditions, CVE studies can include incomplete schedules, mixed vaccine products and varying proportions of SARS-CoV-2 variants circulating. Settings

of CVE studies can be varied and include study populations of COVID-19 patients admitted to hospital, healthcare worker populations, residents, and workers of long-term care facilities, as well as outpatient settings [15-18]. Each setting provides a particular piece of information on the effectiveness of COVID-19 vaccines.

CVE studies among people presenting at primary care/outpatient level generally provide information on how well the COVID-19 vaccines perform against less severe disease. This information complements that of CVE against severe outcomes and is an important factor for public health decision making around vaccine policy. Long-term monitoring of CVE and impact in a variety of settings is crucial to understand the value of the vaccines as a public health intervention and provides important information for future vaccine composition.

## 1.2 Context: influenza

In 2009, the Council of Ministers recommended that all EU Member States reach an influenza vaccination coverage of 75% in key target groups (such as older people, healthcare workers and individuals at risk of more severe disease) [19]. A survey performed by ECDC, reviewing coverage in the three seasons between 2018 and 2021, concluded that although in the 2020–21 season there was generally increased uptake, the recommended target was not reached in most countries [6]. In the same survey, it was also documented that during 2021–22 season, several EU/EEA countries expanded their influenza vaccine recommendations to additional age groups compared to previous seasons especially in children/adolescents and some lowered the lower age limit in elderly adults as compared to 2017/18.

Influenza viruses undergo frequent genetic and antigenic changes. As a consequence, the influenza vaccine is reformulated each year and annual revaccination is recommended. The available vaccine products, the target groups for vaccination and vaccination coverage all vary across countries. The available influenza vaccines are wide-ranging and can be trivalent or quadrivalent, adjuvanted or non-adjuvanted, live attenuated or inactivated, egg-based or cell-based, split virion or subunit vaccines etc. Due to this variation, IVE comparisons by vaccine type (adjuvanted vs non-adjuvanted, live attenuated vs inactivated, egg- vs cell-based), group (split virion, subunit, etc.) and product (vaccine brands) would provide essential information for vaccine recommendations and health economic assessments.

Observed IVE varies from year to year, between population subgroups (e.g. age and risk groups) and varies according to the measured outcome (laboratory confirmed influenza virus by (sub)type/clade or clinical outcome). IVE may vary between vaccine types and products, by time since vaccination and according to previous influenza infection and influenza vaccine history.

Conducting annual IVE estimates at the European level right at the beginning of a seasonal influenza epidemic/pandemic may provide critical and additional evidence for public health recommendations. For example, the recommended target groups for the current season's vaccine can be expanded/amended if circulating strains are causing severe illness but there are good/high early IVE estimates. Alternatively, very low early IVE estimates could prompt recommendations to use other non-pharmaceutical interventions (such as use of face masks or social distancing) in specific settings, or antivirals as a preventative measure. Lacking early season IVE results could also result in a failure to use alternative measures, leading to increased disease burden and increased costs.

Monitoring the IVE along the course of the epidemic/pandemic allows more precise estimates of the impact of current vaccination strategies on the burden of disease to support vaccination campaigns, and may trigger further investigations on seasonal and pandemic vaccines (improve composition, use of adjuvants, different doses, need for booster doses). Additionally, IVE studies can counterbalance the reports of adverse events following immunisation by providing elements for adequate risk management and cost-effectiveness analysis.

Early-season IVE by product and influenza type/subtype is also critical for the World Health Organization (WHO) vaccine composition meeting discussions, in which the vaccine selections for the subsequent season are decided.

Furthermore, new vaccines are being developed for which limited or no effectiveness data are yet available in the EU. In case of an influenza pandemic, an established EU platform to rapidly measure IVE by vaccine type and product will allow the evaluation of any pandemic vaccine and the adaptation of preventive and control strategies.

Besides measuring IVE by influenza (sub)type early and late in the season and by vaccine type, these IVE studies can be used to help answer further research questions, such as the effect of previous influenza vaccination on current season influenza infection, to what extent IVE may decline within the season, the effect of first influenza infection on subsequent IVE and the measurement of IVE against different influenza clades or genetic variants.

Currently, observational studies are the most frequently used method to provide estimates of IVE in Europe. Long-term multicentre studies may provide adequate sample size and data quality to answer some of these questions.

### 1.3 Context: integrated respiratory surveillance

Across Europe, it is recommended that surveillance for respiratory pathogens (including both influenza and SARS-CoV-2) should be integrated [20-22]. Effective integrated respiratory surveillance systems should provide data sufficient for monitoring the spread and intensity of respiratory viruses to guide control measures and mitigate their impact. These systems will also be important in the event of future pandemics. In this context, well-designed, representative sentinel surveillance systems in primary and secondary care should remain the central surveillance method for acute respiratory infections. Sentinel systems provide robust epidemiological data that are routinely collected using common syndromic case definitions with reliable denominators and integral microbiological testing that can be extended to multiple viruses. This makes them ideal as the basis of integrated impact assessment of influenza, COVID-19, and potentially other respiratory virus infections. Monitoring systems should be sensitive enough to detect virus variants, accurately follow virus-specific disease incidence by level of severity/age/place, and assess vaccine effectiveness.

Further information on integrated respiratory surveillance can be found in the [Operational considerations for respiratory virus surveillance in Europe \(18 July 2022\)](#).

### 1.4 ECDC COVID-19 and influenza vaccine effectiveness studies

In 2018, the Council recommendation on Strengthened Cooperation against Vaccine-preventable Diseases (2018/C 466/01) called on the European Commission to work with the Member States and with the support of the European Medicines Agency (EMA) and in cooperation with ECDC to 'continuously monitor the benefits and risks of vaccines and vaccinations at EU level including through post-marketing authorisation studies'. In 2020, the European Commission stressed the importance of continuously monitoring the safety and effectiveness of vaccines in the EU/EEA in the post-authorisation phase, with particular emphasis on COVID-19 vaccines in the context of the ongoing pandemic [7]. This was subsequently formalised as part of the extended EMA regulatory mandate [9] and ECDC's newly amended mandate [10], which request the two Agencies to develop a structured and independent post-authorisation vaccine monitoring platform, initially prioritising COVID-19 vaccines. ECDC and EMA officially established and launched a platform in May 2022, with the intention of bringing together public health and regulatory experts to discuss the studies needed to generate real-life evidence on the safety and effectiveness of vaccines in use in EU/EEA immunisation programmes.

Already in 2020, utilising the lessons learned from other vaccine effectiveness studies, ECDC started building infrastructure to perform COVID-19 vaccine effectiveness (CVE) studies (Table 2). The purpose of this infrastructure is to build a system to regularly monitor VE and perform studies in different settings, and depending on the setting, to provide information on different outcomes (severe disease, moderate disease, infection, transmission, etc). The multi-country approach is also one of the key features that characterises the studies, with a foreseen approach of progressive inclusion of more countries over time.

**Table 2. Settings and types of study performed within the ECDC VEBIS infrastructure as of July 2023**

Setting	Type of study	Main outcome	Pathogens included	Protocol/s
Hospitals	Test negative design	Severe disease (~SARI)	Influenza and COVID-19	[23]
Healthcare workers cohort	Prospective cohort study	Infection	COVID-19 (pilot: Influenza)	[24, 25]
Electronic healthcare databases	Retrospective cohort study	Hospitalisation, death	COVID-19	[26]
Primary care	Test negative design	Moderate disease (~ARI/ILI)	Influenza and COVID-19	<i>This document</i>

## 1.5 Aim of the core protocol

This document presents the core protocol for ECDC studies of CVE and IVE against symptomatic laboratory-confirmed influenza or SARS-CoV-2 infection, respectively, at primary care level. This core protocol presents the main elements for a multicentre (multi-country) study of IVE/CVE at primary care level, outlining the agreed methods for collecting data related to influenza and SARS-CoV-2 at country level, and includes a plan for the pooled analysis. The combination of data from multiple study sites will allow for studies with more statistical power to meet more specific objectives. The protocol can be implemented for either COVID-19 or influenza, or ideally both.

The proposed method is a case-control study using a test negative design. The study population consists of people of all ages, belonging to the target group for COVID-19 or influenza vaccination, presenting to primary care with symptoms of acute respiratory infection (ARI) or influenza-like illness (ILI), and no contraindication for being vaccinated with the vaccine of interest.

This protocol is adapted from the I-MOVE and I-MOVE+ generic protocols [1, 2] and the I-MOVE-COVID-19 generic protocol [3] and is written in a generic manner. The specificities of each study site's IVE and/or CVE study can be detailed in the individual site protocol annexes (Annex 5).

This core protocol, therefore, is primarily intended to guide the implementation of ECDC-funded studies. However, ECDC encourages the implementation of VE studies, using this protocol as a basis, in countries not currently planning to participate in ECDC-funded studies. The use of consistent protocols will facilitate the comparability of study results across studies, countries, and study sites.

This document presents version 1.0 of the core protocol which is an evolving document. This document will be updated and revised as necessary.

This core protocol is complemented by a questionnaire: a list of variables to be collected and their coding are available in Annex 1.

Under each paragraph, arrow marks with italicised text indicate the points that countries/study sites could further expand when creating a country-specific protocol using the core ECDC protocol.

Protocols for IVE and/or CVE studies in other settings and part of the VEBIS project, such as in hospitals or in long-term care facilities, are also available on [ECDC's website](#).

## 2 Objectives

### 2.1 Primary objective

The primary objective is to measure, for each EU/EEA primary care study site participating in IVE and CVE<sup>1</sup> studies, and for pooled analyses, the direct effect (effectiveness) of influenza and COVID-19 vaccines overall and by vaccine product against symptomatic laboratory-confirmed influenza or SARS-CoV-2 infection, respectively.

### 2.2 Secondary objectives

The secondary objectives are to measure overall and product-specific IVE and CVE against symptomatic laboratory-confirmed influenza or SARS-CoV-2 infection, by:

- risk groups (such as clinically vulnerable people, pregnant women, etc.);
- sex;
- age groups;
- specific vaccination target groups;
- calendar time;
- time since vaccination;
- vaccine schedules;
- delay between doses (if more than one dose received);
- combination of vaccine products (if applicable).

Secondary objectives include also identifying key phenotypic or genotypic evolutions that could affect vaccine performance and to estimate IVE and CVE against specific influenza (sub)clades or SARS-CoV-2 genetic variants.

- *Each study site/country to specify primary objectives of their study as measuring CVE and IVE or CVE only or IVE only.*
- *Each study site/country to specify the (additional) secondary objectives of their study.*

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<sup>1</sup> Countries only participating in CVE can ignore references to measurement of IVE throughout the document and vice versa.

## 3 Methods

### 3.1 Study design

At study site/country level: test-negative, case-control study design in primary care setting.

At European level: test-negative, case-control study design in primary care setting using pooled data from several countries.

### 3.2 Study population

The study population comprises community-dwelling individuals who present to participating physicians in the primary care setting, with symptoms meeting the case definition of their influenza or COVID-19 surveillance system, with no contraindications for influenza vaccination (for IVE) or COVID-19 vaccination (for CVE).

- *Study sites/countries to describe the setting of the influenza and COVID-19 surveillance for IVE and CVE studies (number of primary care practices included, number of primary care physicians, catchment population if possible).*
- *Study sites/countries to describe target group(s) for vaccination and order/timing of vaccination (by group when known).*
- *Study sites/countries to describe the epidemiological situation (incidence, number of influenza or COVID-19 cases).*

### 3.3 Study period

The study period for seasonal IVE starts 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<sup>2</sup>. Cases and controls are included from the week of onset of the first influenza positive case presenting in each country-specific study.

The study period for CVE starts when the COVID-19 vaccine is available for the target group of interest in each of the participating countries and when SARS-CoV-2 is circulating. The study period is defined for each priority vaccination group, and begins for each vaccination group, when vaccination campaign in this group begins.

Physicians in participating primary care practices recruit patients and collect data throughout the year.

- *Study sites to define the beginning of the study period for the IVE/CVE study (date/month/year).*
- *Each study site specifies the date of the start of their influenza/COVID-19 vaccination campaign for each priority vaccination group, for the vaccine products used, including recommendations for delay between first, second and subsequent doses (if applicable).*

### 3.4 Outcomes

#### IVE studies

The primary outcome of interest will be PCR laboratory-confirmed influenza in symptomatic patients of all ages consulting at primary care level. The specific outcomes of interest are:

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

#### CVE studies

The primary outcome of interest will be PCR laboratory-confirmed SARS-CoV-2 in symptomatic patients of all ages consulting at primary care level. Confirmation with rapid-diagnostic tests can be considered if highly specific and sensitive tests are used (see section 3.6 on 'laboratory methods').

Secondary outcomes of interest, in the same patient group at primary care level, will be genetic variants of SARS-CoV-2.

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<sup>2</sup> The end of the study period could be defined as the week before two consecutive weeks of no influenza cases after the peak.

## 3.5 Case and control definitions

Case definitions for surveillance can vary between systems in different countries and regions. The case definitions for suspected influenza cases and COVID-19 cases in this protocol are suggestions and can be adapted to the study site-specific surveillance protocols. Collecting detailed symptom information allows reconstitution of case definitions among data collected, if initial case definitions are sensitive.

### 3.5.1 General case definitions

Within the EU, standard surveillance case definitions have been established for influenza-like illness (ILI) or acute respiratory infection (ARI) [20].

An ILI patient is defined as an individual who consults a participating physician, presenting with sudden onset of symptoms AND at least **one** of the following four systemic symptoms:

- Fever or feverishness
- Malaise
- Headache
- Myalgia

AND at least **one** of the following three respiratory symptoms:

- Cough
- Sore throat
- Shortness of breath

An ARI patient is defined as an individual who consults a participating physician, presenting with sudden onset of symptoms AND at least **one** of the following four respiratory symptoms:

- Cough
- Sore throat
- Shortness of breath
- Coryza

AND a clinician's judgment that the illness is due to an infection.

### 3.5.2 Case definitions for IVE study

A **suspected influenza case** will be a patient meeting the EU ILI case definition or the EU ARI case definition [20].

A **confirmed influenza case** in the context of this study will be defined as a suspected influenza case with a respiratory sample PCR-positive for influenza virus. Only symptomatic patients (suspected influenza cases) are included.

An **influenza negative patient (a test-negative control)** will be defined as a suspected influenza case with a respiratory sample PCR-negative for influenza virus.

In the context of integrated influenza and COVID-19 surveillance, either an ILI or an ARI case definition can be used to select patients for swabbing and, if symptoms are collected with high quality, in the analysis the symptom information can be used to restrict to patients meeting the EU ILI case definition.

### 3.5.3 Case definitions for CVE study

A **suspected COVID-19 case** will be someone meeting the EU ARI case definition [20].

A **confirmed COVID-19 case** in the context of this study will be defined as a suspected COVID-19 case with a respiratory sample PCR-positive for SARS-CoV-2. Only symptomatic patients (suspected COVID-19 cases) are included.

A **COVID-19 negative patient (a test-negative control)** will be defined as a suspected COVID-19 case with a respiratory sample PCR-negative for SARS-CoV-2.

Other case definitions could be considered in the context of the study site-specific respiratory pathogen surveillance.

- *Each study site to indicate which case definitions they will use for the IVE and CVE study.*

### 3.5.4 Other control groups (optional)

In addition, an analysis with different control groups will be carried out. The default control group will be those patients testing PCR-negative to influenza (for IVE studies) or SARS-CoV-2 (for CVE studies). Other control groups will include (where this information is available):

- Patients testing PCR-negative to all respiratory viruses.
- Patients testing PCR-positive to respiratory viruses other than influenza (if IVE) or SARS-CoV-2 (if CVE), for example: rhinovirus, RSV, etc (noting that patients with seasonal coronaviruses are excluded for CVE).
- Patients attending general practitioner (GP) practices for reasons other than a respiratory infection (a traditional case control study) could also be considered, particularly during periods where there may be low circulation of other respiratory viruses (more relevant for CVE).

In the context of a high correlation between influenza and COVID-19 vaccination, control groups can exclude SARS-CoV-2 PCR-positive patients (IVE) or influenza PCR-positive patients (CVE), see section 5.1.7.

## 3.6 Laboratory methods

Primary care practitioners will collect respiratory specimens from either all or a systematic sample (see section 3.7.1) of eligible patients (meeting the case definitions above and consenting to take part in the study), respecting safety standards for influenza and COVID-19 and following WHO biosafety guidelines<sup>3</sup>. Depending on the setting, some practitioners will refer patients to specific testing centres or medical laboratories.

It would be beneficial if those countries only measuring CVE test for both SARS-CoV-2 and influenza during the influenza season, as well as for all other respiratory viruses (as appropriate depending on time of year), if possible. If this is not feasible, then during influenza season all samples that are negative for SARS-CoV-2 should also be tested for influenza.

- *Each study site to describe the type (nasopharyngeal/oropharyngeal or both) and number of swabs taken for each patient.*
- *Each study site to describe where swabbing will be carried out (at practice, at home, in centres, a mixture).*
- *Each study site measuring only CVE to indicate which testing strategy they will use (testing all samples for both SARS-CoV-2 and influenza, or only testing for influenza in those negative for SARS-CoV-2).*
- *Each study site to indicate whether they can test for other respiratory viruses, or only SARS-CoV-2 and influenza, or only SARS-CoV-2.*

Quality control tests should systematically be run using PCR to ensure presence of human cells in the respiratory specimens. In the absence of cells, a negative result should be considered inconclusive and a second swabbing should take place if possible.

The ECDC-recommended influenza and SARS-CoV-2 laboratory confirmation is by viral RNA detection with nucleic acid amplification tests, such as RT-PCR. Isolates will undergo molecular analysis for currently circulating SARS-CoV-2 virus.

Information will be collected on type of test.

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 all isolates, or a random sample thereof. The sample should be random and thus be representative of cases and be large enough to provide reasonable precision when calculating proportions of virus change over time.

Gene sequences should also be uploaded to GISAID's open access EpiFlu (influenza) or EpiCoV (SARS-CoV-2) platform. The GISAID EpiFlu/EpiCoV accession number can be provided alongside the epidemiological data, or stored separately along with a unique identifier for the epidemiological data. If random selection of viruses to sequence is used, the proportion sequenced may vary over time, according to a variety of factors, including resources and incidence of influenza virus or SARS-CoV-2. Study sites should indicate their sampling fraction for sequencing over time (see Annex 2, Table 9). Processed genetic information, e.g. name of genetic clade, can also be included within the epidemiological database.

- *Each study site to describe the laboratory procedures (samples taken, storage, transport).*

<sup>3</sup> Any non-propagative diagnostics (e.g. sequencing, RT-PCR) should be conducted at a facility using procedures equivalent to biosafety level 2 (BSL-2), while propagative work (e.g. virus culture, isolation, or neutralisation assays) should be conducted at a containment laboratory with inward directional airflow (BSL-3). Patient specimens from suspected or confirmed cases should be transported as UN3373, 'biological substance category B'. Viral cultures or isolates should be transported as category A, UN2814, 'infectious substance, affecting humans'. World Health Organization (WHO). End-to-end integration of SARS-CoV-2 and influenza sentinel surveillance: revised interim guidance, 31 January 2022. Available at: <https://apps.who.int/iris/handle/10665/351409?locale-attribute=pt&>



- *Each study site to describe the tests and the kits used (and their sensitivity, specificity, positive predictive value) for influenza virus and SARS-CoV-2 and, if needed, other respiratory virus detection.*
- *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 methods for genetic and, when it becomes available, antigenic characterisation.*
- *Each study site to describe genetic and, when it becomes available, antigenic analyses and specify sequencing methods, as well as describing numbers of viruses not sequenced due to CT values or other technical reasons (see Annex 2, Table 9).*

## 3.7 Study participant identification

### 3.7.1 Selection of patients to swab

Study participants are patients with symptoms compatible with suspected COVID-19, as described in section 3.5, consulting a participating GP. Consultation is defined as:

- Having a face-to-face consultation with the GP (in the practice or at the patient's home);
- Having a telephone/video consultation with the GP<sup>4</sup> and then having a swab collected soon after the consultation, either by:
  - A referral to a dedicated COVID-19/respiratory testing centre;
  - A referral to a medical laboratory/specimen collection centre;
  - Having a swab collected by a physician or nurse:
    - At the GP's practice;
    - At a patient's home.

Following the procedures outlined by each study, all or a systematic sample of patients are selected and asked to provide a swab specimen for laboratory testing. Sampling all suspected influenza or COVID-19 cases is preferred, in particular all patients aged 65 years and over for influenza. If this is not possible, then a systematic sample with known sampling fraction can be taken, e.g. the first three suspected cases seen each week per GP, including all patients aged 65 years and over.

- *Each study site to describe the procedures to select suspected influenza and COVID-19 cases to swab.*
- *Each study to describe how/where/by whom swabs will be collected.*

### 3.7.2 Patient inclusion criteria

Patients are eligible if they meet the inclusion definition and consent to participate (the patient or her/his legal guardian gave consent to participate according to the local ethical review process).

- *Each study site to describe country procedures for oral informed consent or written informed consent and specify these in the study annexes.*

### 3.7.3 Patient exclusion criteria

Patients are **excluded from the primary analysis** if they:

- refuse to participate in the study;
- are not swabbed;
- are unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons;
- cannot be swabbed due to severe septum deviation, obstruction or other conditions that contra-indicate swabbing;
- have contraindications for the influenza (for IVE) or COVID-19 vaccination (for CVE);
- are institutionalised (virus exposure and risk factors may be different – specific cohort studies can be undertaken in these groups);
- are swabbed more than seven days (IVE) or 10 days (CVE) after symptom onset (to avoid false negatives; other numbers of days can be explored in sensitivity analyses);
- were vaccinated with a two-dose vaccine with fewer days between dose 1 and 2 than recommended by the manufacturer;
- had an inconclusive RT-PCR test.
- for IVE:
  - patients who had tested positive before to any influenza virus in the current season;
  - patients who had received antivirals 2 weeks prior to swabbing;

<sup>4</sup> In certain circumstances, e.g. if COVID-19 testing centres are physician led, a consultation with a GP is not needed prior to the swab.

- for CVE:
  - patients who had tested previously positive to SARS-CoV-2 within 60 days prior to the current illness episode (other numbers of days and scenarios where date of previous test is unknown will be explored in sensitivity analyses);
  - controls positive to a seasonal coronavirus (e.g. HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1) (as they may be affected by the vaccination).

Reasons for exclusion are documented.

Study sites can collect information on the exclusion factors and exclude patients according to available evidence on these factors.

In the main analysis, patients will be excluded if they were vaccinated with their most recent vaccine dose within 1–13 days preceding symptom onset (see section 3.8). In secondary analyses by time since vaccination, these patients could be included.

In sensitivity analyses, study sites can carry out the VE analyses with different cut-offs of numbers of days between onset and swabbing, using (for example) 5 and 7 days (see section 3.13.2).

In further sensitivity analyses, patients reporting testing previously positive to SARS-CoV-2 will be included, and if sample size allows, an analysis stratified by previous infection will be carried out. Additionally, if sample size allows, patients testing previously positive to SARS-CoV-2 will be included or excluded with varying days since last infection, or variant of infection, if this information is available.

Please see section 3.13.2 on sensitivity analyses.

### 3.7.4 Restriction to priority groups for vaccination for IVE and CVE

IVE will be estimated in the influenza vaccine target group, although as many people have the opportunity to receive influenza vaccine outside the designated target groups, IVE in the general population will also be estimated.

For CVE, patients will be included in the analysis if they are part of a target group for COVID-19 vaccination (particularly booster vaccination), at time of swab. Different vaccine target groups can be used, such as target group for primary course vaccination, or target group for booster vaccination. For some of these target groups, e.g. target group for booster vaccination, country-specific recommendations of time between last dose and current dose need to be taken into account (e.g. 3 or 5 months since last vaccination). As many people have had (and will continue to have) the opportunity to receive COVID-19 vaccine outside the designated target groups, CVE in the general population will also be estimated.

## 3.8 Exposure (vaccination)

### 3.8.1 Definition of vaccination status

#### *Current seasonal influenza vaccine*

An individual is considered as:

- vaccinated against influenza if they received one dose of the (product-specific) current season influenza vaccine 14 or more days before disease onset;
- unvaccinated if they
- unvaccinated if they:
  - did not receive influenza vaccine in the current season; or
  - received one dose of the (product-specific) current season influenza vaccine less than 14 days before disease onset; or
  - were vaccinated after their symptom onset date.

#### *Current pandemic COVID-19 vaccine*

COVID-19 vaccination rollout schedules are evolving and this section will need to be updated on an ongoing basis to reflect any new vaccination schedules.

An individual will be considered as vaccinated against COVID-19 with a product-specific vaccine under the following categories:

- **Fully vaccinated with a primary course, first booster plus second booster:** Patients will be considered fully vaccinated with second booster if they have **received completed primary course vaccination plus booster (see below) followed by a second booster dose** at least 14 days\* before symptom onset.
- **Fully vaccinated with a primary course plus booster:** Patients will be considered fully vaccinated with booster if they have **received completed primary course vaccination (see below) followed by a booster dose** at least 14 days\* before symptom onset.

- **Fully vaccinated with a primary course** (two-dose vaccine): Patients will be considered fully vaccinated if they have **received both doses** at least 14 days<sup>5</sup> before symptom onset.
- **Fully vaccinated with a primary course** (single-dose vaccine): Patients will be considered fully vaccinated if they have **received one dose** at least 14 days<sup>5</sup> before symptom onset.
- **Partially vaccinated** (two-dose vaccine only): Patients will be considered partially vaccinated if they have **received one of two doses** at least 14 days<sup>5</sup> before symptom onset.
- Patients will be considered as **unvaccinated** if they **did not receive COVID-19 vaccine** or if they **were vaccinated after onset** of symptoms.

As the COVID-19 vaccine schedules evolve, collecting detailed vaccination information including dates of each individual vaccination may become complicated and burdensome, particularly when the data source for vaccine information is not electronic. As a minimum, study sites should collect information on number of doses of COVID-19 vaccine and date and brand of last vaccine received. It is highly recommended to collect the brands of each COVID-19 vaccine received if possible, most importantly the brand of the first dose (to differentiate between primary course with single-dose vs two-dose vaccines) and the brand of the last dose.

- *Each study site to describe the country COVID-19 vaccination recommendations and schedule, including specific recommendations by age/at-risk groups, and policy regarding timings between doses of primary course and booster doses, as well as the timing of vaccine rollout.*
- *Each study site to describe the vaccination variables they are collecting.*

### 3.8.2 Vaccination status ascertainment

The exposure of interest in this study is a vaccination history with influenza vaccine or COVID-19 vaccine. The vaccination history includes date of administration, vaccine product and brand name, and the number of doses received (if applicable). Documenting the batch codes (where this is feasible) will allow identification of the vaccine brand, the vaccine content and the dose.

An individual is considered as vaccinated if they meet at least one of the following criteria:

- they are registered as vaccinated in a vaccination registry (preferred option);
  - they self-report having received an influenza or COVID-19 vaccination;
  - they are registered as vaccinated against influenza or COVID-19 in the GP information system;
  - their insurance company can show evidence of pharmacy delivery or re-imburement of influenza or COVID-19 vaccine/vaccination;
  - influenza or COVID-19 vaccination has been recorded in their vaccination card/vaccination booklet.
- *Each study site to document:*
- *the vaccine products used;*
  - *places of vaccination (GPs, specific vaccination centres, etc.);*
  - *the precise mode of vaccine ascertainment (self-report, card, registry, etc.);*
  - *if no precise dates of vaccination are collected, the variable allowing a patient to be defined as vaccinated or unvaccinated;*
  - *vaccine status ascertainment validation;*
  - *potential limitations of the vaccine status ascertainment.*

## 3.9 Data to be collected, including potential effect modifiers and confounding factors

### 3.9.1 Patient characteristics

Study sites can document the following patient characteristics to describe the study population:

- Age in years;
- Sex;
- GP code (in order to account for clustering by GP);
- Smoking history: never smoked, former smoker (stopped smoking for at least one year), current smoker (including stopped smoking less than one year ago). Smoking refers to any type of smoking (cigarettes, cigars, vaping, etc.);
- Pregnancy (yes/no).

<sup>5</sup> The exact number of days will depend on the vaccine; this number may change and the protocol will be updated when more information is available.

### 3.9.2 Information on consultation

Type of consultation if different types of consultations are used: in practice, video, telephone, home, at a COVID-19 centre.

### 3.9.3 Clinical signs and symptoms

Collection of good quality symptom information is crucial for the VE study in order to be able to validate the case definition used (see section 3.5). As a minimum, if only CVE is estimated, data on the following symptoms should be collected:

- sudden onset;
- cough;
- fever;
- shortness of breath;
- coryza.

If IVE is estimated, the following **additional** variables should be collected to be able to determine which patients meet the EU ILI case definition:

- headache;
- sore throat;
- myalgia;
- malaise.

Study sites should collect the **date of symptom onset**.

➤ *Study sites to document the symptoms collected in their IVE and CVE studies.*

### 3.9.4 Information on swabbing and test results

For each patient, sites will collect information on:

- date of swabbing;
- place of swabbing (if relevant): GP practice, medical laboratory, COVID-19 centre, self-swabbing;
- type of influenza or COVID-19 test (PCR, rapid test);
- result of influenza or COVID-19 test.

Study sites carrying out testing for other respiratory viruses should also collect test results from any other respiratory viruses (e.g. rhinovirus, RSV, enterovirus, adenovirus, human metapneumovirus, seasonal coronaviruses, etc).

### 3.9.5 Pre-existing chronic conditions

If physicians are recruiting cases and controls using electronic medical records, the list of ICD codes can be used to document a study participant's chronic diseases (see Table 3):

The list below is comprehensive. A suggested minimum number of chronic diseases is specified below.

**Table 3. ICD-10 codes for chronic diseases**

Category	ICD-10
Anaemia	D50-D53, D55-64
Asplenia	Q89.0, Q20.6, Z90.8
Asthma	J45
Chronic liver disease	K70, K72-74, K75.4, K76.9
Cardiovascular diseases	A52.01, B37.6, B58.8, I05-9, I11, I13, I20-25, I26.0, I26.9, I27, I30-51, I97.0-1, R00.1, T81.7, T82.8, Q20-24, Q25.1-2, Q26.0-1, Q26.8, Q87.4, R01.1-2
Diabetes	E10-11
Hypertension	I10, I15.8, I15, I15.1, I15.2, I97, I27.0
Obesity	E66.01, E66.2, E66.9
Immunodeficiency* or organ transplant	B20, D80-84, D89.8-9, Z21, Z94
Neuromuscular disorders	G70.0, G70.2, G70.8, G70.9, G73.7
Renal disease	M10.3, N00-N08, N10-19, N20.0, N28.9
Dementia	F01, F03, F05, G30, G31, G91, G94
Stroke	G93, I67.8, I69
Rheumatologic diseases	M30-34, M35.0, M35.5, M35.8-9, M05-06, M08, M12.0
Cancer	C00-96
Lung disease excluding asthma)	A15, J40-44, J46-47, J60-J70, J80-86, J90-J94, J96, J99, J182, M34.8, M05.1
Tuberculosis	A15-A19

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

If ICD codes are not available, a list of underlying conditions should be prepared by using a short questionnaire.

The list of underlying conditions in the questionnaire should include chronic conditions that are associated with an influenza (IVE) or COVID-19 (CVE) vaccination recommendation if possible:

- diabetes (study sites are encouraged to distinguish between type 1 and type 2);
- cardiovascular disease: myocardial infarction, angioplasty, coronary artery bypass surgery, stroke, transient ischemic attacks, treated hypercholesterolemia, not including hypertension;
- hypertension;
- chronic pulmonary disease (not including asthma);
- asthma;
- cancer;
- renal disease;
- chronic liver disease;
- rheumatologic diseases;
- obesity (see paragraph below);
- immunodeficiency<sup>6</sup>.

For obesity, if body mass index (BMI), or height and weight are not available, study sites can collect the following categories: BMI=30–39 and BMI $\geq$ 40.

### 3.9.6 Vaccination status – vaccines other than influenza/COVID-19

If desired, study sites can collect information on vaccination against pneumococcal, RSV (once available) and/or other relevant vaccinations. In particular, RSV vaccination may be of relevance for IVE/CVE studies if recommended to similar target groups as influenza/COVID-19 and uptake is sufficiently high. Vaccination status variables could include:

- disease (pneumococcal/RSV/other);
- vaccination status;
- type of vaccine;

<sup>6</sup> This can include active or recent treatment for solid tumour and hematologic malignancies; receipt of solid-organ or recent hematopoietic stem cell transplants; severe primary immunodeficiency; advanced or untreated HIV infection; active treatment with high-dose corticosteroids, alkylating agents, antimetabolites, tumour-necrosis (TNF) blockers, and other biologic agents that are immunosuppressive or immunomodulatory.

- number of doses;
- either date or year of vaccination;
- vaccination status ascertainment/source.

See section 3.8.2 on vaccination status ascertainment for information on methods for vaccination ascertainment (provided for COVID-19/influenza but applicable to other vaccines).

➤ *Each study site/country to document other vaccination status variables collected in their IVE and CVE studies.*

### 3.9.7 Antiviral administration

The use of antivirals should be documented: type, dosage (if possible) and date of administration (patients receiving antivirals in the two weeks prior to swabbing will be excluded from IVE analysis), as this may lead to misclassification biases.

➤ *Each study site/country to document antiviral variables collected in their IVE and CVE studies.*

### 3.9.8 Previous influenza or SARS-CoV-2 infection

If information on previous influenza infection within the current influenza season is known, this can be documented and controls with this infection will be excluded from IVE analyses.

Among those patients consulting their GP with COVID-19-like symptoms, some may have already had a SARS-CoV-2 infection in the past. People who have been previously infected may have a greater response to the vaccine or be less likely to be reinfected even if unvaccinated. If possible, study sites will collect the following information:

- whether the patient had a previous positive SARS-CoV-2 test(s) (yes/no/unknown);
- optional:
  - type(s) of test: PCR, rapid test, serology (in case of multiple positive test results, the most recent);
  - date(s) of test (in case of multiple positive test results, the most recent);
  - history of COVID-19, e.g. clinical confirmation.

In the primary analysis, controls testing SARS-CoV-2 positive in the previous 60 days will be excluded from the analysis. Sensitivity analyses will be carried out including controls who previously tested SARS-CoV-2 positive, and stratifying by previous SARS-CoV-2 infection, if sample size allows. Additionally, if sample size allows and information is available, controls previously infected with SARS-CoV-2 will be included or excluded depending on differing days since last infection, or variant of infection.

### 3.9.9 Previous tests used for the same illness episode

In order to better understand healthcare seeking behaviour and impact of home-testing, information on previous tests used for the current illness episode can be collected. This is applicable to the CVE study, as home tests are not routinely available for influenza.

This could be in form of the questions:

- Have you already done a test for SARS-CoV-2 for this illness episode?
- What type of test was it (PCR, rapid test)?
- What were the results (positive, negative, unknown)?

In the sensitivity analyses, patients testing positive at home for the current illness episode and negative in the study can be excluded from the analysis.

### 3.9.10 Healthcare utilisation in the previous 12 months

In order to document and control for healthcare seeking behaviour in the control groups and the severity of underlying conditions, study sites can collect:

- the number of GP visits made (face-to-face, or telephone consultations) in the past 12 months before inclusion in the study;
- the number of hospital admissions due to underlying conditions in the 12 months prior to inclusion in the study.

## 3.10 Sample size

The number of people included in the VE study will depend on the number of patients consulting at primary care level with ARI or ILI (depending on case definitions used) and the number of patients PCR-confirmed with influenza (IVE) or SARS-CoV-2 (CVE). Sample size will also depend on the duration of the period under study. The pooled analyses should not prevent study teams from including a big enough sample size to obtain reasonably precise estimates for each study site-specific analyses. Sample size estimation for VE studies differs from that for

hypothesis testing. We are more concerned with the precision of the VE estimate than with determining whether this estimate is significantly different from the null. For example, the confidence interval around a high VE estimate (e.g, 70%) may exclude 0% and provide little information (e.g. if the lower bound is equal to 1%). Better would be to aim for a more precise VE estimate (e.g. with a lower boundary of, say, 50%). Conversely, the confidence interval around a low VE estimate (e.g. 5–10%) may include 0% and still be informative. Besides, aiming for confidence intervals excluding 0% when the VE is low may result in unreasonably large sample sizes.

The following sample size estimates focus on the precision of the VE estimate (Table 4). As mathematically 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 90% (in 20% increments), varying vaccine effectiveness with the OR between 0.2 and 0.7 (equivalent to VE between 80% and 30%). The sample size for IVE and CVE may differ, as VE, vaccine coverage and positivity rates will vary. Sample size estimates should be estimated for IVE and for CVE and the higher estimates should be used.

A dynamic version of this table in Excel sheet format is available for study sites on request.

**Table 4. Sample size calculations**

Precision of 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	1 346	1 346	50	40–58
0.1	1	0.6	0.3	1 764	1 764	40	30–49
0.1	1	0.7	0.3	2 240	2 240	30	20–39
0.3	1	0.1	0.5	32	32	90	60–98
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

Precision of lower CI boundary	Controls/case	Detectable OR	Vaccine coverage in source population/controls	Number of cases	Number of controls	VE	CI
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	1 337	1 337	40	30–49
0.1	1	0.7	0.5	1 751	1 751	30	20–39
0.3	1	0.2	0.7	43	43	80	50–92
0.3	1	0.3	0.7	71	71	70	40–85
0.3	1	0.4	0.7	108	108	60	30–77
0.3	1	0.5	0.7	153	153	50	20–69
0.3	1	0.6	0.7	207	207	40	10–60
0.3	1	0.7	0.7	272	272	30	0–51
0.2	1	0.2	0.7	75	75	80	60–90
0.2	1	0.3	0.7	131	131	70	50–82
0.2	1	0.4	0.7	205	205	60	40–73
0.2	1	0.5	0.7	298	298	50	30–64
0.2	1	0.6	0.7	412	412	40	20–55
0.2	1	0.7	0.7	548	548	30	10–46
0.1	1	0.2	0.7	219	219	80	70–87
0.1	1	0.3	0.7	413	413	70	60–78
0.1	1	0.4	0.7	676	676	60	50–68
0.1	1	0.5	0.7	1 015	1 015	50	40–58
0.1	1	0.6	0.7	1 435	1 435	40	30–49
0.1	1	0.7	0.7	1 941	1 941	30	20–39
0.3	1	0.2	0.9	71	71	80	50–92
0.3	1	0.3	0.9	129	129	70	40–85
0.3	1	0.4	0.9	208	208	60	30–77
0.3	1	0.5	0.9	310	310	50	20–69
0.3	1	0.6	0.9	437	437	40	10–60
0.3	1	0.7	0.9	591	591	30	0–51
0.2	1	0.2	0.9	124	124	0.8	60–90
0.2	1	0.3	0.9	238	238	0.7	50–82
0.2	1	0.4	0.9	397	397	0.6	40–73
0.2	1	0.5	0.9	605	605	0.5	30–64
0.2	1	0.6	0.9	868	868	0.4	20–55
0.2	1	0.7	0.9	1 190	1 190	0.3	10–46
0.1	1	0.2	0.9	361	361	0.8	70–87



Precision of lower CI boundary	Controls/case	Detectable OR	Vaccine coverage in source population/controls	Number of cases	Number of controls	VE	CI
0.1	1	0.3	0.9	751	751	0.7	60–78
0.1	1	0.4	0.9	1 311	1 311	0.6	50–68
0.1	1	0.5	0.9	2 061	2 061	0.5	40–58
0.1	1	0.6	0.9	3 022	3 022	0.4	30–49
0.1	1	0.7	0.9	4 216	4 216	0.3	20–39

CI: confidence interval; OR: odds ratio; VE: vaccine effectiveness.  
Sample sizes calculated using Stata™'s power functionality.

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 is planned.

## 3.11 Data

### 3.11.1 Datasets and coding

Some study sites may not be able to collect all information proposed above. Study sites can indicate which variables they can collect and which data source they will use in Annex 1: 'List of variables collected, definition and coding'. Study sites can use the coding (variable names and values) as specified in Annex 1 or use their own coding. For the pooled analysis, if study site-specific coding is used, a data dictionary can be supplied alongside the data.

### 3.11.2 Data collection instruments

Data will be collected using a standardised questionnaire/data collection form. Some information may require follow-up. The source(s) of data may include:

- face-to-face/telephone interview;
- electronic medical records;
- interview with patient or their family;
- vaccination and other registries;
- laboratory records.

➤ *Each study site to define the sources of information used for each variable collected (see also Annex 1).*

### 3.11.3 Data collection validation

If paper questionnaires are being used, a sample of paper questionnaires will be checked against the study database to validate data entry.

For GPs using electronic medical records, a sample of patients can be asked to fill a questionnaire and questionnaire results can be compared to the medical records and the study database.

➤ *The specific validation procedures, including sample size calculation for questionnaire validation (if applicable) should be specified in the study annexes. Vaccination status, date, dose(s) if relevant and vaccine brand should be collected carefully and validated.*

## 3.12 Data management

### 3.12.1 Data collection, entry, and storage at study site level

Web-based data collection methods or paper-based methods can be used. Data entry procedures will include checks to minimise data entry errors.

Laboratory information will be reported to the surveillance site coordinator using the reporting procedures existing in each study site for influenza and COVID-19 surveillance.

Information on antigenic, when available, and genetic analyses, including GISAID accession number can be included in the epidemiological database or stored separately on an Excel spreadsheet (see Annex 2).

All data should be stored and processed in a way compliant with GDPR.

- *Study sites to specify procedures of data collection and data entry.*
- *Study sites to specify methods of data storage and their compliance with the GDPR requirements.*
- *Study sites to provide a data dictionary (codebook) that includes the variable names, variable descriptions, and the coding of variable values (see also Annex 1).*

## 3.13 Analysis

### 3.13.1 Individual (country/study site level) analysis

The timing to conduct such analyses will depend on the epidemiological situation and the sample size achieved. Example scripts can be provided if desired, and support is available for analysis.

Cases and controls will be described by baseline characteristics. Patients will be described according to:

- sex;
- age group;
- time: month of symptom onset;
- influenza (IVE) or COVID-19 vaccination status (CVE);
- absence, presence of at least one, or presence of more than one high-risk condition;
- specific chronic conditions (e.g. diabetes, immunodeficiencies, respiratory diseases, cardiovascular diseases);
- pregnancy;
- vaccination status of other vaccines;
- respiratory co-infections.

An example layout of this descriptive analysis is provided in Table 5 below.

**Table 5. Example of descriptive table for cases and controls; COVID-19/influenza vaccine effectiveness study at primary care level, country X, year**

Variables	Number of laboratory-confirmed COVID-19 (or influenza) cases/total n (%)	Number of test-negative controls/total n (%)
<b>Median age (IQR)</b>	X	X
Missing	X	X
<b>Age groups (years)</b>		
0–14	x/x (x)	x/x (x)
15–45	x/x (x)	x/x (x)
45–64	x/x (x)	x/x (x)
65–79	x/x (x)	x/x (x)
≥80	x/x (x)	x/x (x)
Missing	X	X
<b>Sex</b>		
Female	x/x (x)	x/x (x)
Missing	X	X
<b>Days between onset of symptoms and swabbing</b>		
0	x/x (x)	x/x (x)
1	x/x (x)	x/x (x)
2	x/x (x)	x/x (x)
3	x/x (x)	x/x (x)
4–7	x/x (x)	x/x (x)
8–10 (if CVE)	x/x (x)	x/x (x)
<b>COVID-19/influenza vaccination status</b>	x/x (x)	x/x (x)
Missing	x	X
<b>Etc.</b>		

In a second step, a univariable analysis will be carried out to measure the association between vaccination and being a laboratory-confirmed influenza or COVID-19 case. This study is a case-control study (test-negative design). The measure of association is an odds ratio (OR). This can be estimated by logistic regression. An OR = 1 indicates no association between an exposure and the outcome. An OR > 1 indicates a potential risk factor, an OR < 1 indicates a potential protective factor, noting that the confidence interval around the OR helps with its interpretation.

For vaccination as preventive factor, the IVE/CVE can be computed as  $IVE/CVE = (1 - OR) * 100$ . The logistic regression model computes a 95% confidence interval around the point estimate of the OR, and the same formula as above can be used to obtain the VE 95% confidence interval.

A crude VE for the IVE/CVE against being a laboratory-confirmed influenza/COVID-19 case will be estimated.

A stratified analysis (by sex and age group, for example) can follow to better understand potential effect modifiers and confounders.

Prior to multivariable analysis, a model development strategy should be determined (see also Annex 4). Creating direct acyclic graphs may help better understand how the variables relate to each other and the outcome. In a final step, a multivariable analysis will be carried out to take confounding factors and potential effect modifiers into account. Please see Annex 4.

### Output tables presenting IVE/CVE estimates

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 6 below 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 6. Example of table displaying vaccine effectiveness against COVID-19/influenza adjusted for various covariables overall and by age group; COVID-19/influenza vaccine effectiveness study at primary care level, country X, year**

Clade/variant	Population included	Analysis scenarios/adjustments made	CVE (or IVE) (%)	(95%CI)
COVID-19 (or influenza)	All ages	N (cases/vaccinated; controls/vaccinated)		
		Crude		
		Adjusted for onset date (cubic spline)		
		Adjusted for sex		
		Adjusted for chronic condition		
		Adjusted for age (cubic spline)		
		Adjusted for onset date, age (cubic spline)		
		Adjusted for onset date, chronic condition		
		Adjusted for onset date, age (cubic spline), chronic conditions, sex		
		0–49 years	N (cases/vaccinated; controls/vaccinated)	Crude
Adjusted for onset month, age (cubic spline)				
50 years and over	N (cases/vaccinated; controls/vaccinated)			Crude
		Adjusted for onset date, age (cubic spline), chronic condition, sex		

### 3.13.2 Sensitivity analyses

As sensitivity analyses, study sites can measure VE:

- with different cut-offs of numbers of days between onset and swabbing;
- with different cut-offs of numbers of days between vaccination and onset of symptoms;
- varying the time period post-vaccination (e.g. seven days, 14 days, 15-30 days, 30-59 days, etc.) to be considered 'fully vaccinated' or 'partially vaccinated' (see section 3.8.1);
- including, excluding and stratifying by those with previous positive tests, and also with different delays between previous test and inclusion in the study;

- excluding patients testing positive at home as part of the current illness episode and testing negative as part of the study;
- using only controls positive to other respiratory viruses;
- excluding controls testing SARS-CoV-2 positive (IVE) or influenza positive (CVE), or adjusting by COVID-19 (IVE) or influenza (CVE) vaccination (see also section 5.1.7);
- including those fully vaccinated but with inappropriate gaps between doses.

### 3.13.3 Pooled analysis

The higher sample size for the pooled analysis will provide more precision around the VE estimates. Data can be coded as outlined in Annex 1, or a data dictionary can be provided by the study teams that includes the variable names, descriptions and coding. The central hub (performing the pooled analysis) will perform data cleaning and document and share any further data cleaning and analysis with all study coordinators to ensure it can be reproduced.

See Annex 4 for detailed guidelines for analysis, including the pooled analysis. For the pooled data, interim analyses will be conducted in different periods if appropriate and 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 influenza (for IVE) or COVID-19 (for CVE), vaccination coverage, the recruitment strategy and the number of participating primary care practices.

The pooled analysis will be carried out in a similar way to the study site-specific analysis. Country or study site will be included as a fixed effect or potentially as a random effect in a multilevel model. Statistical heterogeneity between study sites will be determined, using the Q-test and the  $I^2$  index.

## 3.14 Personal data protection

Each study site conducting the study shall comply with requirements stemming from data protection legislation, and with national ethics committee requirements. Where required, informed consent will be sought from all participants or legal guardians. The national ethics committees will specify whether oral, written, or no consent will be required. A copy of the ethical approvals should be sent to the coordinating centre.

Ethical approval and consent forms should state that the patient's data will be used for a multicentre study and results will be shared with the scientific community (articles, presentations, etc.).

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

## 3.15 Safety

During consultations, during the swabbing procedure and during handling of biological specimens, the safety of the practitioners is paramount. Any person swabbing, handling swabs and swabbing material, should be fully trained and ensure that adequate personal protective equipment is used and hygiene measures followed, according to national and clinic-specific guidelines.

- *Each study site to state the safety measures carried out, related to swabbing, handling of biological specimens, transport of samples, authorisation levels of biologists, biosafety levels of laboratories, etc.*

## 3.16 Training

Investigators and data collectors will be trained on the study protocol before the start of the study. They will receive the protocol and questionnaires.

The target audience and material for training will depend on the data collection methods (e.g. GP interviews, electronic database usage) and the dataflow structures in place. This could include training videos, paper-based training material and practice visits. Special emphasis should be put on the systematic selection of patients to swab, in terms of importance and logistics.

- *Each study site to describe the training to be organised.*

## 4 Logistical aspects

### 4.1 Study site leader

In each study site, a study site leader will coordinate the study at the country level and act as focal point for a pooled analysis.

### 4.2 Human resources

In each study site, an investigator will be in charge of monitoring data collection at the GP office level. GPs will collect the information among consulting patients. The specific human resources needed in each country are detailed in the study annexes.

## 5 Limitations

Any observational study is subject to an array of biases, some of which are outlined below. Other limitations include, in the context of a new SARS-CoV-2 variant causing more severe disease or a very severe influenza season, that primary care systems may become disrupted or overloaded, making data collection difficult.

### 5.1 Potential biases

#### 5.1.1 Bias from pooled estimates

With data from study sites from different countries being pooled, any bias in the individual studies will influence the pooled estimate. The power of the test for the presence of heterogeneity between individual studies is low if there are few sites/countries. In this case, the test may not be able to detect heterogeneity between them, despite it being present. It is important that heterogeneity is also 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 association between influenza/COVID-19 vaccination and the outcome.

There are many conditions which could lead to bias in a study site. For CVE, being a novel virus, there are evolving surveillance systems and strategies in each participating country. There are different tests being used (PCR and rapid antigen tests), potentially for different age groups and with changes over time, which may impact how the SARS-CoV-2 outcome is defined (particularly if rapid antigen tests are used for defining the SARS-CoV-2 outcome). Additionally, home testing may affect who sees a GP for an ARI or ILI episode, as knowing their SARS-CoV-2 status may affect whether people consult their GP (which may be differential by vaccination status).

To allow for complete assessment of heterogeneity, study sites need to document all changes in their influenza and COVID-19 surveillance system/s during the study period.

- *Each country to document any changes in influenza/COVID-19 surveillance during the study period.*

#### 5.1.2 Negative confounding

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

#### 5.1.3 Positive confounding

Positive confounding refers to biases that reflect a 'healthy vaccinee effect'. People with a healthy lifestyle will be more likely to accept vaccination, and may be less likely to become infected, thus leading to an increase of measured CVE/IVE. People with risk-taking behaviours may also be averse to vaccination, which may also increase their exposure to disease. If positive confounding is present, IVE/CVE will be overestimated.

#### 5.1.4 Unmeasured confounding

Positive and negative confounding will be minimised through stratification and multivariable analysis. The test-negative design used here may help overcome some of the unmeasured/difficult to measure confounding. Unmeasured confounding could include heterogeneity of exposures among unvaccinated and vaccinated (the distribution of high-risk behaviours may differ between these groups). 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, as in any observational study, some residual unmeasured confounding may remain.

- *Each study site to describe the potential limitations and representativeness of the subjects included.*

#### 5.1.5 Previous infection in cases or controls

People who have been previously infected may have a stronger response to the vaccine or be less likely to be reinfected even if unvaccinated. It is possible that some of the controls (those testing negative for influenza/SARS-CoV-2) may have themselves been positive for influenza/SARS-CoV-2 some time before. The proportion of these (potentially immune individuals) in each country's dataset would depend on the circulation of the virus in the community in the months before the patient presented to the GP. Knowledge of their prior infection could affect their likelihood to be vaccinated. For example, if someone knew that they had had COVID-19, despite having no symptoms (e.g. if they had had a screening test), they may be subsequently less likely to be vaccinated. This would lower vaccination coverage among controls and decrease CVE. Similarly, if someone believed that they had

had influenza but had not had a test, they may be subsequently less likely to be vaccinated. This would lower vaccination coverage among controls and increase IVE.

Ascertainment of which controls may have had previous influenza/SARS-CoV-2 infection can be attempted by asking about previous influenza/SARS-CoV-2 tests and results, as well as prior clinical symptoms. However, there could potentially be several patients with prior SARS-CoV-2 or influenza infection, unbeknownst to them. Results should be interpreted in light of this, and an estimate of a range of potential bias should be calculated around the IVE and CVE estimates. Sensitivity analyses should be conducted including any patients with previous influenza (IVE) or SARS-CoV-2 (CVE) infection confirmed either by PCR or by serological tests.

### 5.1.6 Validation of exposure

The vaccination status is the exposure of interest, and therefore reliable vaccination data are critical. If the vaccination status is only self-reported, without written or electronic documentation, information bias may occur. Vaccination status of cases and controls should ideally be validated using an independent source (i.e., vaccination register, GPs database, patient's medical record).

### 5.1.7 Inclusion of influenza-positive controls for CVE and SARS-CoV-2-positive controls for IVE

A key prerequisite of the test-negative design is that the vaccination of interest does not affect the control group. However, there may be a strong correlation between those receiving influenza and COVID-19 vaccine. If this is the case, inclusion of SARS-CoV-2-positive controls may artificially lower the vaccine coverage among influenza test-negative controls, biasing towards a lower IVE. Conversely, inclusion of influenza-positive controls may artificially lower the vaccine coverage among SARS-CoV-2 test-negative controls, biasing towards a lower CVE. The test-negative design is no longer valid.

To account for this, analyses should be carried out excluding SARS-CoV-2-positive controls (IVE) and influenza-positive controls (CVE), if adequate information is available [27]. These estimates can be compared to the primary analysis of including SARS-CoV-2-positive/influenza-positive controls. An alternative method to account for this potential bias is to adjust by COVID-19 vaccination (IVE) or influenza vaccination (CVE) if this information is available [27].

Sensitivity analyses will be conducted excluding controls who are positive for SARS-CoV-2 from IVE estimation, and excluding controls who are positive for influenza from CVE estimation.

### 5.1.8 Other potential biases

Controls could come from different source populations with varying risk for infection with influenza or SARS-CoV-2, varying probability for acquiring influenza or COVID-19 vaccination, etc. (e.g. depending on time of year). Time (onset date) will be used to adjust for seasonal differences. Analyses will also be stratified by time (e.g. onset quarter). For COVID-19, the success of vaccination programs in some countries has resulted in only a small proportion of population remaining unvaccinated. This population may be different from the general population. Rather than using unvaccinated as the reference group in CVE, analyses using relative VE (e.g. booster vaccination compared to primary course vaccination) could be considered, although a simulation study is recommended to understand the implications of this analysis in a multicentre context.

- *Each country to describe timeline of vaccination and vaccine coverage for different target groups.*

## 5.2 Representativeness of subjects included in the study

The study includes cases that are consulting GPs for influenza-like or COVID-19-like symptoms. Containment and mitigation strategies for the COVID-19 pandemic may differ by country depending on the case management strategy (e.g. recommendation of contacting a specific COVID-19 helpline, or consulting a GP or health centre by telephone first). In some cases, the management strategy will have an impact on which patients consult a GP and are swabbed. This also may have an impact on the time lag between the onset of symptoms and respiratory specimen collection, and currently we do not know if this may affect false negativity rates. Beside the collection of the aforementioned data in the protocol, case-containment/mitigation/healthcare-seeking strategies should be described for each country. Note that the test-negative design adjusts for case management strategies, e.g. patients with contact to a confirmed case, as both cases and controls come from this population.

Some countries will have parallel diagnostic pathways, e.g. patients with respiratory symptoms self-referring to COVID-19 centres and patients being swabbed by GPs. This could cause a lack of representativeness among those participating in the study. This will have the most impact on VE if this is differential by case and vaccination status.

There may be country-specific recommendations on rapid antigen test use for SARS-CoV-2, which may vary by different age groups and across different time periods. If rapid antigen tests are used in the study for determining

case status, the recommendations for use should be described and sensitivity analyses excluding patients tested with rapid antigen test carried out.

With the widespread use of rapid antigen tests, it may be that patients consult a GP to confirm a positive rapid antigen self-test. Conversely, it may be that patients consult a GP only if the rapid antigen self-test was negative. If rapid antigen test positivity is associated with vaccination status, the CVE may be biased.

In certain age groups, particularly older age groups, vaccination coverage is extremely high, reaching over 95%. Such high coverage, besides affecting sample size, renders the representativity of unvaccinated controls questionable. In this situation, analyses using relative VE (e.g. booster vaccination compared to primary course vaccination) could be considered (see 5.1.8).

- *Each study site to describe the potential limitations in terms of representativeness of the subjects included.*
- *Each study site to describe the recommendations of rapid antigen test use over time and by age group (if relevant). Study sites to describe the availability of rapid tests and any recommendations around PCR confirmation of tests.*



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## Annex 1. List of variables, definitions, and coding: minimum dataset

The following list of variables constitutes the proposed dataset of an IVE/CVE study at primary care level. Variables in green are optional. Study sites may not be able to collect all the proposed data and can list the variables collected in the study-specific annex.

Study sites can follow this variable naming and coding, or are welcome to code variables and values in their own way.

- *Study sites can use Table A1 below to indicate which variables they are collecting and data sources.*
- *Study sites to indicate all modifications in the variables collected and coding compared to variables below.*

**Table A1. List of variables, including values, coding, and definitions**

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
<b>Study-related variables</b>				
participate	<input checked="" type="checkbox"/>	Numeric (binary)	0 = No 1 = Yes	Agrees to participate
id	<input checked="" type="checkbox"/>	Type of variable at discretion of site	[needs to be unique]	Unique and persistent identifier for each record
gpcode	<input checked="" type="checkbox"/>	Type of variable at discretion of site	[needs to be unique]	Unique identifier for each GP
<b>Optional</b>				
refuse	<input type="checkbox"/>	Text		Reasons for refusal to participate
<b>Demographics</b>				
age <sup>7</sup>	<input type="checkbox"/>	Numeric (continuous)	Integer	Age of each participant in years
sex	<input type="checkbox"/>	Numeric (binary)	0 = female 1 = male	Sex of study participant
<b>Signs and symptoms</b>				
onsetdate	<input type="checkbox"/>	Date	dd/mm/yyyy	Date of onset of symptoms
fever	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Fever or feverishness
cough	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cough
shortbreath	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Shortness of breath
malaise	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes	Malaise

<sup>7</sup> If age in years not possible, please provide age in 5-year age groups.

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
			8 = Do not know	
myalgia	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Myalgia
sorethroat	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sore throat
suddenonset	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sudden onset
headache	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Headache
coryza	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Coryza or rhinitis
<b>Optional</b>				
anosmia <sup>8</sup>	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Anosmia (Loss of sense of smell)
ageusia <sup>9</sup>	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Aguesia/dysgeusia (Loss or distortion of sense of taste)
<b>Swabbing/testing information</b>				
swabdate	<input type="checkbox"/>	Date	dd/mm/yyyy	Swabbing date
test_type	<input type="checkbox"/>	Numeric (categorical)	1 = PCR 2 = Point of care/rapid antigen test 3 = Other 8 = Do not know	Type of test used (if other, please specify)
lab_sarscov2	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Inconclusive 8 = Do not know	Laboratory result for SARS-CoV-2 (positive/negative)
lab_flu	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Inconclusive 8 = Do not know	Laboratory result for influenza (positive/negative)
lab_virusa	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus type A

<sup>8</sup> Anosmia and ageusia can also be combined into 'anosmia-ageusia'.

<sup>9</sup> See above.

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
lab_virusb	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus type B
lab_h1n1	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus subtype AH1N1
lab_h3n2	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: virus subtype AH3N2
byamagata	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: B Yamagata lineage
bvictoria	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result: B Victoria lineage
<b>Optional</b>				
swabplace	<input type="checkbox"/>	Numeric (categorical)	1 = GP practice 2 = COVID-19 centre 3 = Self-swabbing 4 = Swab at home by healthcare worker 8 = Do not know	Place of swabbing (if relevant)
swab_type	<input type="checkbox"/>	Numeric (categorical)	1 = Nose 2 = Throat 3 = Both nose and throat 4 = Saliva 8 = Do not know	Type of swab taken
test_thisepisode	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Have you already been tested for SARS-CoV-2 as part of this illness episode?
testtype_thisepisode	<input type="checkbox"/>	Numeric (categorical)	1 = PCR 2 = Point of care/rapid antigen test 3 = Other 8 = Do not know	If yes to above: What test type were you tested with (for this illness episode)?
testres_thisepisode	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	What was the test result (for this illness episode)?
gisaidid_sarscov2	<input type="checkbox"/>	Text		GISAID accession number for SARS-CoV-2

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
gisaidid_flu	<input type="checkbox"/>	Text		GISAID accession number for influenza
geneticvariant_sars cov2	<input type="checkbox"/>	Text		Genetic variant of SARS-CoV-2 virus using Pango nomenclature (can be collected separately at different date)
clade_flu	<input type="checkbox"/>	Text		Genetic variant of influenza virus (can be collected separately at different date)
<b>Results for other respiratory pathogens (Optional, but RSV and seasonal coronavirus are recommended)</b>				
lab_rsv <sup>3</sup>	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for RSV (positive/negative)
lab_metapneum	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for human metapneumovirus (positive/negative)
lab_rhinovirus	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for rhinovirus (positive/negative)
lab_adenovirus	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for adenovirus (positive/negative)
lab_bocavirus	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for bocavirus (positive/negative)
lab_seascorona <sup>4</sup>	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for seasonal coronavirus (positive/negative)
lab_enterovirus	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for enterovirus (positive/negative)
lab_parainfluenza	<input type="checkbox"/>	Numeric (categorical)	0 = Negative 1 = Positive 2 = Not done 8 = Do not know	Laboratory result for parainfluenza (positive/negative)

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
<b>Vaccination variables</b>				
covvaccany	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	COVID-19 vaccination status (any vaccination)
covvaccany_firstdose	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	COVID-19 vaccination status of first dose
covvaccany_seconddose	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	COVID-19 vaccination status of second dose
covvaccany_thirddose	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	COVID-19 vaccination status of third dose
covvaccany_4	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	COVID-19 vaccination status of fourth dose
covvaccdoses	<input type="checkbox"/>	Numeric (categorical)	0 = 0 doses 1 = 1 dose 2 = 2 doses 3 = 3 doses 4 = 4 doses 5 = 5 doses 99 = Do not know	COVID-19 vaccine doses
covvaccddate_firstdose	<input type="checkbox"/>	Date	dd/mm/yyyy	COVID-19 vaccination date of first dose
covvaccddate_seconddose	<input type="checkbox"/>	Date	dd/mm/yyyy	COVID-19 vaccination date of second dose
covvaccddate_thirddose	<input type="checkbox"/>	Date	dd/mm/yyyy	COVID-19 vaccination date of third dose
covvaccddate_4	<input type="checkbox"/>	Date	dd/mm/yyyy	COVID-19 vaccination date of fourth dose
covvaccddate_last <sup>5</sup>	<input type="checkbox"/>	Date	dd/mm/yyyy	COVID-19 vaccination date of last dose
covvacbrand_firstdose	<input type="checkbox"/>	Text		Brand name of first dose COVID-19 vaccine
covvacbrand_seconddose	<input type="checkbox"/>	Text		Brand name of second dose COVID-19 vaccine
covvacbrand_third	<input type="checkbox"/>	Text		Brand name of third dose COVID-19 vaccine

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
covvacbrand_4	<input type="checkbox"/>	Text		Brand name of fourth dose COVID-19 vaccine
covvacbrand_last <sup>6</sup>	<input type="checkbox"/>	Text		Brand name of last dose COVID-19 vaccine
fluvaccany	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination in current season
fluvaccdate	<input type="checkbox"/>	Date	dd/mm/yyyy	Influenza vaccination date
fluvaccype	<input type="checkbox"/>	Text		Type of vaccine (brand name)
fluvacc_n1	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination in previous season
<b>Optional</b>				
fluvacc_n2	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received flu vaccination in season before previous season (i.e. current – 2)
<b>Underlying chronic conditions</b>				
diabetes	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Diabetes and endocrine disease
heart_dis	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Heart disease
immuno	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Immunodeficiency and organ transplant
lungdis	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic lung disease
obese	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = BMI $\geq 30$ 8 = Do not know	
<b>Optional</b>				
chronany_inf	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Has a chronic condition that is associated with an influenza vaccination recommendation.
chronany_cov	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Has a chronic condition that is associated with a COVID-19



Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
				vaccination recommendation.
heart_disnohyp	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Heart disease (excluding hypertension)
hyperten	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Hypertension
lungdis_noasthma	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic lung disease excluding asthma
asthma	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Asthma
cancer	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cancer
renal_dis	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Renal disease
liver_dis	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Liver disease
rheum_dis	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Rheumatological disease
<b>Possible exclusion criteria</b>				
antivir	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Administration of antivirals prior to swabbing
res_home	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Living in a residential home
contra	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Contra-indication for COVID-19 or influenza vaccination
prevtest_cov	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Has the patient had any positive SARS-CoV-2 test prior to this illness episode?
<b>Optional</b>				
antivirdate	<input type="checkbox"/>	Date	dd/mm/yyyy	Date administration antiviral
antivirtype	<input type="checkbox"/>	Text		Type of antiviral (brand name)

Variable name	Collected by study site? (Please also indicate data source if not patient interview)	Type	Values and coding	Definition
prevtest_type_cov	<input type="checkbox"/>	Numeric (categorical)	1 = PCR 2 = Rapid test 3 = Serology 3 = Other (please specify) 8 = Do not know	Type of test used to detect a previous episode of SARS-CoV-2
prevtest_date_cov	<input type="checkbox"/>	Date	dd/mm/yyyy	Date of most recent positive SARS-CoV-2 test prior to this illness episode?
prevtest_flu	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Has the patient had a positive influenza test in the current influenza season?
<b>Other variables</b>				
gpvisit	<input type="checkbox"/>	Numeric (count)	integer	Number of GP consultations in the previous 12 months
pregnant	<input type="checkbox"/>	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Pregnancy status
<b>Optional</b>				
severity	<input type="checkbox"/>	Numeric (count)	integer	Number of hospitalisations in the previous 12 months for the chronic disease
smoking	<input type="checkbox"/>		0 = Never 1 = Former 2 = Current 9 = Do not know	Never, former (stopped smoking at least 1 year before inclusion in the study), current smoker (any smoking can be included: cigarettes, cigars, vaping, etc.)

1. If age in years not possible, please provide age in five-year age groups.

2. Anosmia and ageusia can also be combined into 'anosmia ageusia'.

3. Optional, but highly desirable.

4. Optional, but highly desirable.

5. Study sites that are unable to provide dates of each vaccination can provide number of doses and the date and brand of last dose. It is highly recommended to also provide the brands of all doses (even if dates are not collected), or at a minimum just the brand of the first and last doses.

6. Study sites that are unable to provide brand of each vaccination can provide number of doses and the date and brand of last dose. It is highly recommended to also provide the brands of all doses (even if dates are not collected), or at a minimum just the brand of the first and last doses.

## Annex 2. Genetic information (examples)

Information related to genetic sequencing can be included in the epidemiological database, or provided separately in a spreadsheet, as below. The minimum amount of data needed to obtain genetic data from GISAID (sequences of all viruses should be sent to GISAID's open access EpiCoV platform) is ID number and GISAID accession number. Additional information on CT value, selection for characterisation, and reasons for not characterising can be additionally collected (see Table A2).

**Table A2. Example of a data collection form for genetic data**

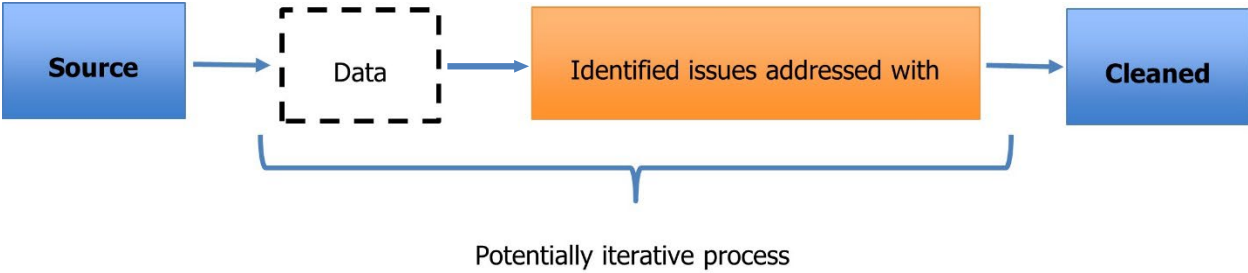
	Unique ID number	GISAID accession ID number	Selected for characterisation?	Reasons for not characterising?	CT value	Type of sample (primary specimen or isolate)
Strain 1						
Strain 2						

Where not all viruses were attempted to be sequenced, but only a random selection of them, additional information on sampling fraction should be provided. An example can be seen in Table A3.

**Table A3. Example of documenting how viruses were selected for sequencing over time**

Time period	First date of time period	Last date of time period	Sampling fraction used	Date used for definition of time unit (onset date, swab date, other)	Comments
<i>Example1</i>	<i>01/10/2020</i>	<i>31/12/2020</i>	<i>1</i>	<i>Date of onset</i>	<i>(this is only an example; all specimens were characterised)</i>
<i>Example2</i>	<i>01/01/2021</i>	<i>15/02/2021</i>	<i>0.2</i>	<i>Date of onset</i>	<i>(this is only an example; 20% of all specimens were characterised)</i>

# Annex 3. Data flow for pooled dataset



## Annex 4. Detailed pooled analysis plan

Each individual study site can analyse their data. Example scripts can be provided if desired, and support is available for analysis. The following analysis plan covers the pooled analysis.

### Descriptive analysis

The proportion of eligible hospitalised cases and controls who accepted to participate in the study will be calculated. The proportion of patients not consenting will be documented, along with reasons for no participation. Patients excluded will be described in a study flowchart.

Cases and controls will be described by baseline characteristics (Table A4).

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

- number of GPs/GP centres participating and catchment population;
- beginning of vaccination campaigns for COVID-19 and for influenza vaccines;
- definitions of target groups for vaccination;
- beginning and end of the study period;
- vaccine product(s) used; and
- estimated vaccine coverage in the country/region by vaccine brand, by target vaccine group.

**Table A4. Example for CVE (or IVE) of descriptive table for cases and controls**

Variables	Number of laboratory-confirmed COVID-19 (or influenza) cases/total n (%)	Number of test-negative controls/total n (%)
<b>Median age (IQR)</b>	X	X
Missing	X	X
<b>Age groups (years)</b>		
0–14	x/x (x)	x/x (x)
15–44	x/x (x)	x/x (x)
45–64	x/x (x)	x/x (x)
65–79	x/x (x)	x/x (x)
≥80	x/x (x)	x/x (x)
Missing	X	X
<b>Sex</b>		
Female	x/x (x)	x/x (x)
Missing	X	X
<b>Days between onset of symptoms and swabbing</b>		
0	x/x (x)	x/x (x)
1	x/x (x)	x/x (x)
2	x/x (x)	x/x (x)
3	x/x (x)	x/x (x)
4–7	x/x (x)	x/x (x)
8–10 (if CVE)	x/x (x)	x/x (x)
<b>COVID-19/influenza vaccination status</b>	x/x (x)	x/x (x)
Missing	x	X
<b>Etc.</b>		

Patients will be described according to:

- sex;
- age group;
- healthcare worker status;
- time: month of symptom onset;
- symptoms;
- high-risk condition: absence, presence of at least one, presence of more than one
- specific chronic conditions (e.g. respiratory, cardiovascular diseases);
- pregnancy;
- COVID-19 and influenza vaccination status;
- respiratory co-infections; and
- study site.

## Measure of effect

This study is a case control study (test-negative design). The measure of association is an odds ratio (OR). This can be measured by logistic regression. An OR = 1 indicates no association between an exposure and the outcome. An OR > 1 indicates a potential risk factor, an OR < 1 indicates a potential protective factor, noting that the confidence interval around the OR helps with its interpretation.

For vaccination as preventive factor, the VE can be computed as  $VE = (1 - OR) * 100$ . A 95% confidence interval is computed around the point estimate.

## Pooled crude VE estimates

We will estimate crude VE, adjusting for study site as a fixed effect and adjusting for symptom onset time. We will adjust for study site, as this is part of the multicentre study design, and for calendar time, as this is an integral part of the test-negative design.

The primary analysis for the pooled VE estimates will be a one-stage approach, where individual data from study sites are pooled and study site is included as a fixed effect covariate in the model. The one-stage analysis is useful approach, particularly if smaller study sites are included and it can provide good modelling flexibility. Study site can be included as a fixed effect or if enough study sites are available potentially as a random effect in a multilevel model. The fixed effect assumes the same VE for all study sites, with differences due to random variation, rather than to essential differences in VE.

Statistical heterogeneity between study sites will be determined, using Q-test and the I2 index [28] and a two-stage pooled analysis approach can be carried out in a sensitivity analysis.

## Pooled stratified analysis

The analysis can be stratified according to (if sample size allows):

- age groups (e.g. <20, 20-44, 45-64, 65+ years);
- sex;
- presence of at least one chronic condition, or specific chronic conditions; and
- calendar time and time since vaccination.

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

## Pooled multivariable analysis

A multivariable logistic regression analysis will be conducted to estimate VE and 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/VE, biological plausibility) will also be used as criteria for inclusion of a variable or an interaction term. As noted in the 'Pooled crude VE estimates' section, study site and onset time should always be controlled for in each model.

## Variable selection and model specification

### *Model development strategy*

To find a suitable model, we will consider very carefully the variables collected and determine which are GP level variables, which are individual level variables, which variables are mediators of each other, and which variables are

potential confounders and effect modifiers. Variables will also be checked for collinearity, and decisions will be made to include the group of collinear variables in the model or select among them.

The above considerations are particularly important for this study, as some of the medication information collected and the chronic conditions of the patients may be strongly correlated.

Creating a direct acyclical graph may help better understand the relation between all variables collected and the outcomes.

Some variables will be a priori variables. These are variables that we want to keep in the model, as previous studies have shown them to be potential confounders or effect modifiers. These could include age and sex, but also potentially others.

If the model is not overfitted and variables are included that are not collinear or mediators, then there may be less concern for parsimony, as including insignificant variables may result in more accurate  $p$ -values for tests for variables of interest.

However, if sample size is low and the model is overfitted, then a backwards step-down variable selection procedure could be considered.

Interaction terms should be included cautiously, factors other than statistical significance (prevalence of exposure, magnitude of OR) will also be used as criteria for inclusion of an interaction term.

Several different models may have to be presented and considered.

### Continuous variables

Continuous variables in the data include age, date of onset of symptoms, and number of GP visits and hospitalisations. 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 model simplicity (so a non-expert can understand what is going on), precision and bias minimisation.

### Output tables presenting VE

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 A5 can be used (variables presented just as an example of the output format). Useful information includes numbers of cases and controls and presentation of results for different models.

**Table A5. Example table of VE for COVID-19 (or influenza), primary care-based VE study**

Clade/variant	Population included	Analysis scenarios/adjustments made	CVE (or IVE) (%)	(95%CI)
COVID-19 (or influenza)	All ages	N (cases/vaccinated; controls/vaccinated)		
		Crude*		
		Adjusted for onset date (cubic spline)		
		Adjusted for sex		
		Adjusted for chronic condition		
		Adjusted for age (cubic spline)		
		Adjusted for onset date, age (cubic spline)		
		Adjusted for onset date, chronic condition		
		Adjusted for onset date, age (cubic spline), chronic conditions, sex		
		0–49 years	N (cases/vaccinated; controls/vaccinated)	
	Crude*			
	Adjusted for onset month, age (cubic spline)			
50 years and over	N (cases/vaccinated; controls/vaccinated)			
	Crude*			
	Adjusted for onset date, age (cubic spline), chronic condition, sex			

\* Symptom onset time and study site is always included in the model.

### 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 odds:

- There are at least 10–15 cases (or controls, whichever is smaller) per study site 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  $\geq 5$  records in each cell of the two-by-two table of case and exposure status.

With low sample size, we should consider collapsing categories, modelling continuous variables in a different way (if applicable). Sensitivity analyses can be carried out using penalised logistic regression.

### Controlling for GP effect

Primary analyses will be carried out using standard logistic regression to obtain the individual study VE estimates. However, there could be variability between GPs. To adjust for this possible cluster effect, a multi-level logistic regression with each GP as a random effect will be carried out and compared to the single level analysis.

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.

## Identifying heterogeneity, testing for heterogeneity

Country-specific crude and adjusted VE 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 healthcare use, and 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 Higgins and Thompson classification, an  $I^2$  index of around 25% indicates low, 50% indicates medium and 75% indicates high heterogeneity between studies [29].

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

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.

### Two-stage pooled analysis approach

A one-stage pooled analysis approach is the primary analysis, and a two-stage meta-analysis approach can be used to obtain the pooled VE estimate in a sensitivity analysis. Study-specific adjusted ORs and standard errors for the effect of different vaccination definitions 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. In a random effects model, the study site-specific estimates are assumed to be normally distributed around a central effect. Thus they are allowed (expected) to differ from each other.



The study-specific exposure-disease effects 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 ( $\tau^2$ ). This will give the pooled odds ratio and standard error (using the DerSimonian Laird approach).

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

In the sensitivity analysis, the two-stage approach can be compared to a one-stage pooled analysis approach, where individual study data are combined and study site is added as a fixed effect (for example).

## Annex 5. Study-specific annexes

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

- The setting of the influenza and COVID-19 surveillance for IVE and CVE studies (number of primary care practices included, number of primary care physicians, catchment population if possible).
- Description of target group(s) for influenza and COVID-19 vaccination and timeline of vaccination by group (when known), for the vaccine products used, including recommendations for delay between COVID-19 doses.
- Description of the epidemiological situation (incidence, number of influenza or COVID-19 cases).
- Definition of the beginning of the study period for the IVE/CVE study (date/month/year), if applicable/possible.
- The case definitions to be used for the IVE and CVE study and the procedures to select suspected influenza and COVID-19 cases to swab.
- Country procedures for oral informed consent or written informed consent and specify these in the study annexes.
- Description of the type (nasopharyngeal/oropharyngeal or both) and number of swabs taken for each patient, as well as where swabbing will take place (at practice, at home, in centres, a mixture).
- Study sites measuring only CVE to indicate which testing strategy they will use (testing all samples for both SARS-CoV-2 and influenza, or only testing for influenza in those negative for SARS-CoV-2).
- Whether testing for other respiratory viruses will be carried out, or only SARS-CoV-2 and influenza (or only SARS-CoV-2).
- Description of the laboratory procedures (samples taken, storage, transport), tests and the kits used (and their sensitivity, specificity, PPV) for influenza virus and SARS-CoV-2 and, if needed, other respiratory virus detection. 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 methods for genetic and, when it becomes available, antigenic characterisation.
- Description of the safety measures carried out, related to swabbing, handling of biological specimens, transport of samples, authorisation levels of biologists, biosafety levels of laboratories, etc.
- Description of the vaccination variables they are collecting. Each study site to document:
  - the vaccine products used;
  - places of vaccination (GPs, specific vaccination centres, etc.);
  - the precise mode of vaccine ascertainment (self-report, card, registry, etc.);
  - If no precise dates of vaccination collected, the variable allowing a patient to be defined as vaccinated or unvaccinated; and
  - vaccine status ascertainment validation.
- Definitions of variables collected and the sources of information used for each variable collected. Study sites to provide a data dictionary (codebook) that includes the variable names, variable descriptions, and the coding of variable values (see also Annex 1).
- Description of procedures of data collection and entry, specification of methods of data storage and their compliance with the GDPR requirements. Study sites to describe how and who performs the database pseudonymisation/anonymisation prior to local data analysis. Each study site to describe the procedures to comply with the national ethics committee requirements and the type of informed consent needed as well as whether consent can be obtained from a legal guardian. Each study site to send a copy of the ethical approval to the coordinating centre.
- Description of the provision of training of GPs.
- Documentation of any changes in influenza/COVID-19 surveillance during the study period.
- Description of the potential limitations and representativeness of the subjects included.
- Description of the recommendations of rapid antigen test use over time and by age group (if relevant). Study sites to describe the availability of rapid tests and any recommendations around PCR confirmation of tests.