



Instituto **Nacional de Saúde**  
*Doutor Ricardo Jorge*



# **Case Control Study for Measuring Influenza Vaccine Effectiveness In Portugal – Final Report**

Season 2010-11

Baltazar Nunes, Pedro Pechirra, Ausenda Machado, Isabel Falcão, Paulo Gonçalves,  
Patrícia Conde, Inês Batista, Raquel Guiomar and José Marinho Falcão.

Instituto Nacional de Saúde Dr. Ricardo Jorge

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## Summary

Every year, influenza virus is responsible for epidemics that affect human health causing respiratory infections that could lead to serious health complications of individuals belonging to risk groups, as well as on the functioning of health services. In order to mitigate influenza impacts, vaccination has been one of the main measures, being recognized its role in reducing the risk of developing the disease and the occurrence of their complications. Thus, since the vaccine is reformulated every season estimating the influenza vaccine effectiveness (VE) every season and in an early stage is of major importance to support public health decisions.

Since 2008-2009, Portugal has been participating in I-MOVE project that aims to estimate seasonal and pandemic vaccine effectiveness during and after the influenza season. Last season, 2010-2011, Portugal once again joined the I-MOVE multi-center case control study (with the national VE study- Euroeva) together with Spain, Ireland, France, Italy, Romania, Hungary and Poland, using a common protocol and with the objective of estimate the 2010-11 seasonal influenza vaccine effectiveness respectively in the elderly (65+) and in all age groups. Additionally, using information on 2010-11 seasonal vaccine coverage in the population, it has been also proposed to estimate 2010-11 seasonal vaccine effectiveness using the screening method.

## Material and Methods

Two different approaches were used so as to estimate vaccine effectiveness:

- a) For the **test negative design (TND)**, a case-control approach was used, where laboratory confirmed influenza cases (ILI+) were compared to laboratory negative influenza ILI patients (ILI-). On a weekly basis, each GP selected systematically ILI patients (two per week from all ages and all ILI patients with 65 years and more) using the EU ILI case definition. Data on confounding factors and effect modifiers was collected using a standardized questionnaire. VE was estimated as one minus the odds ratio of being vaccinated in cases versus controls adjusted for confounders by logistic regression.
- b) For the **screening method**, the 2010-11 seasonal vaccine coverage was compared between a sample of ILI cases and a sample of ILI cases laboratory confirmed for influenza with the vaccine coverage estimated in the general population. ILI cases and ILI Positive cases were the same as the one used in the TND. Vaccine coverage in the population was obtained from a sample of 1074 households stratified by region (homogeneous allocation) selected from a dual sample frame – random digit dialing mobile and landline phones (ECOS sample). The relevant information was collected by

CATI (Computer Assisted Telephone interview) with the same questionnaire – one respondent by household (proxy for the rest of the household members). VE was estimated by comparing the proportion of cases vaccinated to the vaccine coverage in the source population using the Orenstein formula and the Farrington method to adjust for confounders.

For both methods an ILI patient was considered vaccinated if he/she had received one dose of the vaccine at least 14 days prior onset of symptoms. Data analysis comprised ILI positive cases selected between week 45 of 2010 and week 11 of 2011.

## Results

In Portugal, the 2010-2011 influenza season was characterized by a mixed circulation of influenza virus. In the early beginning of the season, B type virus dominated the season, until week 1 where A(H1N1)2009 virus started to dominate. Also in circulation, but in a minor proportion was the A(H3) virus.

### a) Test negative design results:

Considering TND results, 58 GP's accepted to participate in the study, with 60% participation rate (35 GP's effectively participated in the study by selecting patients). Excluding 33 ILI cases (for not meeting the inclusion criteria) the final sample for analyses consisted on 253 ILI patients with a high positive rate (57%). Among cases, 73 were positive for B virus, 69 for A(H1N1)2009 and 2 for A(H3). For analysis purposes three groups of cases were defined: **All influenza**, **Influenza B** and **Influenza A(H1N1)2009** that were compared to ILI cases that tested negative for influenza virus (109 **Controls**). After adjustment for age group, pandemic and seasonal vaccine 2009-10 season, any chronic disease, target group, GP visits and month of onset, VE point estimates were:

- 58% (CI95% -61 – 89) for **All influenza**
- 34% (CI95% -98 – 97) for **Influenza A(H1N1)2009** and
- 75% (CI95% -255 – 88) for **Influenza B**.

Nevertheless, no statistical significance was obtained for either the analysis.

### b) Screening method results

In season 2010-2011, 903 households of the ECOS-sample were interviewed (which corresponds to 2684 individuals). The final estimated vaccine coverage was 17.5% (CI95% 15.1-20.3), with a gradient evolution since September 2010 (3.5%) till December 2010 (16.6%). Crude and adjusted VE (using the Farrington method) estimates were computed for medically attended ILI cases and ILI influenza positive cases. Overall results indicate that after adjustment for confounding (age group and presence of chronic diseases), VE point estimates decreased from 47.0% to 33.3% in ILI cases and from 70.1% to 63.7% in ILI positive cases.

Due to the small sample size, no VE estimates were computed for the individuals of the vaccination target group, i.e., individuals with 65 years and more and the ones with at least one chronic disease.

### **Conclusions**

Overall results obtained by the Euroeva study indicate that crude 2010-11 seasonal VE estimate against medically attended influenza was 79% (CI95% 43-94) and 70% (CI95% 32-87), respectively for the TND and screening method. After adjustment the respective VE estimates decreased: 58 (CI95% -61-89) and 64% (CI95% 17-84). These results were in accordance to the up to now published results (42-65%).

The TND study was also able to provide strain specific 2010-11 seasonal VE estimates: influenza B, crude VE=87% (CI95% 41-99) and adjusted VE=75% (CI95% -98-97) and for influenza A(H1N1)2009, crude VE=74% (CI95% 14-94) and adjusted VE=34% (CI95% -254-88). These results suggest that the 2010-11 seasonal VE was lower than the monovalent A(H1N1)2009 VE estimated by the IMOVE multicenter study in the season 2009-10: 72% (CI95% 46-86).

Our study was unable to estimate VE for specific seasonal vaccine target groups. This result enhances, as in previous studies, the unavoidable need for pooling data from network of VE studies with common protocol as IMOVE.

### **Recommendations**

The main recommendations focused on:

- To calculate sample size taking into consideration:
  - the context of the multicentre case-control study: minimum sample size per site in order to assure a minimum homogeneity for pooled analysis;
  - different expected VE point estimates, i.e. for low, medium and high VE;
  - minimum set of factors for stratified analysis;
  - the adjustment for confounders.
- To increase sample size, mainly in the elderly population (aged 65 years or more);
- To increase the total number of participating GP's in the study by exploring other sources of GP's recruitment;
- To study the inclusion of the population based vaccine coverage uncertainty in the screening method;
- To explore with participating GP's the best way to obtain estimates of the Euroeva ILLI sample fraction.

Finally we also recommend continuing the harmonization of the study designs between participating countries with the multi-centre study objective.



## Introduction

Flu is a serious contagious disease that can lead to hospitalization and even death<sup>1</sup>. Despite influenza virus infection is at the individual level just an unpleasant experience for the majority of the population; the disease impact on European society has important consequences.

In fact, it is estimated by ECDC that up to 40,000 people die each year from influenza in the European Union (EU). On the other side, there are significant costs to the health services of Europe in caring for those sick from influenza and finally there are significant economic impacts deriving from the large numbers of mild to moderate cases which result in time off work and the consequent production losses.

Specific population groups are more at risk of becoming seriously affected by influenza. These are the 'high risk groups' which include people with 65 and more years of age and those with chronic ill-health. For these high-risk groups, yearly vaccination in the autumn is recommended in most EU countries, in an approach known as 'selective vaccination' which reduces their risks of being infected and also develops complications, severe disease and death<sup>2</sup>.

The vaccine is the main method for preventing the disease and its more severe complications. The evaluation, in the same season, of the vaccine effectiveness is of major importance for public health decisions, especially since the vaccine is reformulated every year.

Main conclusions from two pilot studies, with a cohort design, conducted in Portugal by the Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), during the 2005/2006 and 2006/2007 influenza seasons, stressed that estimation of effectiveness of anti-flu vaccine should be based on multicentre studies involving several European countries<sup>3</sup>.

Since 2008-2009, through the Departments of Epidemiology and Infectious Diseases of the Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal has been participating in the project I-MOVE, funded by ECDC, aiming monitoring influenza vaccine effectiveness during influenza seasons and pandemics in the European Union, with the participation of several countries.

Instituto Nacional de Saúde Dr. Ricardo Jorge had previously participated in I-MOVE with the study EUROEVA, a pilot study conducted to test a case-control design able to measure in-season and end of season influenza vaccine effectiveness, during the autumn and winter 2008-2009, among people aged 65 years and above, using several control groups<sup>4</sup>. The study was designed to use preferably routine data provided by the Portuguese system of integrated clinical and virology influenza surveillance, based on the GP Portuguese sentinel network, Médicos-Sentinela. In 2009-10 season, Portugal participated in the I-MOVE multi-center case control study together with Spain, Ireland, France, Italy, Hungary and Romania, using a common protocol and with the objective of estimate the seasonal and pandemic influenza vaccine

effectiveness respectively in the elderly (65+) and in all age groups. At national level the study was unable to provide pandemic and seasonal influenza vaccine effectiveness estimates but contributed with data to the multicentric study<sup>5</sup>.

For the influenza season 2010-2011, Portugal has submitted a new protocol to participate in the I-MOVE, and once again, was selected to participate. This report describes the project development and the results obtained from the data collected from week 45/2010 to week 14 /2011.

## **Objectives**

### **2.1 Primary objectives**

The primary objectives were to measure influenza vaccine effectiveness among people of all ages and those aged 65 years and more in EU/EEA countries.

### **2.2 Secondary objectives**

- To estimate VE in each of the participating countries
- To provide intra-seasonal VE estimates
- To estimate VE by risk group
- To estimate VE by influenza subtype
- To monitor VE estimates every year

## Methods

### Study design

The general design was a case-control approach where laboratory confirmed influenza cases were compared to laboratory influenza-negative ILI patients (test negative design).

### Study population and sampling design

The eligible population for the study included all ILI cases, non institutionalized and resident on the participating GPs catchment area.

The study population was composed of all age's individuals with no contraindication for influenza vaccination.

The sampling was performed in two steps:

1. GPs were contacted and selected from a list of sentinel doctors belonging, or that had belonged, to the MS network. All the GPs of the Portuguese Sentinel Network were invited to participate on the EUROEVA 2010-2011, by ordinary mail and e-mail. Those GPs were also asked to select others to participate on the study, which were contacted later. All GPs that participated on the EUROEVA 2009-2010 were also invited to participate on the current study (EUROEVA 2010-2011).

2. Each GP that accepted to participate was asked to select per week two ILI cases (EU ILI definition) of all ages and all ILI cases from individuals aged 65 years or above from their weekly consults (patient could belong or not to the GP list).

### Study period

In order to estimate seasonal VE, ILI cases were selected by GPs starting on 15<sup>th</sup> November 2010 (week 45). Data collection ended at 10<sup>th</sup> April 2011 (week 14) once since week 11 none of the ILI cases enrolled in the study were positive for influenza (defined previously that 2 weeks with no positive cases for influenza would determine the study end).

Thus, the results presented in this report comprise data collected since the above mentioned starting dates and week 14 of 2011.

### Outcome

A confirmed case of influenza virus infection is defined as a person with an influenza-like illness with laboratory confirmed influenza A(H1N1), A(H1N1)2009, A(H3N2) and B virus

infection by one or more of the following tests:

1. real-time RT-PCR
2. viral culture

### *Case definition*

Influenza-positive ILI cases were considered as **Cases**. A case of influenza like illness (ILI) was defined as an individual who consults a participating GP, presenting a sudden onset of symptoms and at least one of the following four systemic symptoms (EU criteria)<sup>6</sup>:

- fever or feverishness;
- malaise;
- headache;
- myalgia;

AND at least one of the following three respiratory symptoms:

- cough;
- sore throat; and
- shortness of breath.

### *Laboratory confirmation*

#### *Specimens collection*

The success of virus diagnosis largely depends on the quality of the specimen and the conditions for transport and storage of the specimens before it is processed in the laboratory.

Specimens were collected from ILI cases who consult their GP within 7 days after onset of clinical symptoms for influenza like illness.

Nasopharyngeal swabs, or a combined nasopharyngeal with oropharyngeal swab were acceptable. The specimens were collected into a suitable transport medium. This procedure was conducted by the GP himself or by a nurse under his supervision.

Each sample was identified and the information related to the patient, demographic data, characteristics of the disease and the data concerning the confounding variables were recorded on the notification form.

### *Storage, transport*

The specimens on viral transport medium were kept at 0 to 4°C and transferred from the GP to the National Influenza Reference Laboratory by an express mail company within 24 hours, following the procedure already in place for the samples collected for routine surveillance of seasonal influenza.

### *Laboratory Tests (RT-PCR / Culture)*

Laboratory confirmation of influenza infection was done using cell-tissue culture for influenza viruses and a real-time multiplex RT-PCR.

Virus isolation is a very useful technique for the diagnosis of influenza infection allowing for further antigenic and genetic characterization of isolates, and also for vaccine preparation or drug-susceptibility testing.

Isolates were characterized antigenically by haemagglutination inhibition tests (HAI), carried out using antisera and reference virus strains distributed by WHO Collaborating Center (Atlanta). Selected isolates were sent to the WHO Collaborating Center in London for further study.

The rapid detection and (sub)typing of seasonal influenza viruses was performed by a multiplex “in house” real-time RT-PCR targeted to the matrix and nucleoprotein genes of influenza A and B. This is a powerful technique for the identification of influenza virus genomes even when they are present at very low levels.

For influenza A subtyping were used the *Real Time Ready Inf A/H1N1 detection* (Roche) and *ProFlu Influenza A Subtyping* (Prodesse) assays.

In order to identify the influenza B lineage (Yamagata/88 and Victoria/87), a multiplex “in house” real-time RT-PCR was used.

### *Strain characterization*

The phylogenetic analyses of the influenza virus isolates was performed by sequencing the coding region of the HA1 subunit of the haemagglutinin, for a subset of isolates from the beginning, the peak and the end of the season, representing 50% of the isolated strains, using the ClustalW Method for the multiple alignment and the Maximum Likelihood Method for the construction of the phylogenetic trees (MEGA Software).

The reference laboratory follows internal control procedures and external quality control programs organized by Global Influenza Surveillance Network (GISN from WHO) and by European Influenza Surveillance Network (EISN from ECDC).

## *Case finding*

### *Procedures to select ILI cases*

Cases were identified among patients that presented ILI to a participating GP. For the purpose of estimating VE, GPs selected ILI cases individuals of all ages and with 65 years or more. The ILI case could occur among GPs patient list or not, provided that an encounter patient/GP took place.

ILI cases were recruited using the EU case definition, respecting the exclusion criteria (described below) and using a systematic sampling method. This systematic sampling procedure consisted on the selection, by each GP, of the first two ILI cases of all ages of each week and all ILI cases with 65 years or more. To avoid bias regarding the weekday, the first day of the week for each GP was randomly assign (e.g. for GP1 the week starts at Thursday, GP2 Tuesday, GP3 Monday, etc.). In this way, each GP had a different starting day of the week and received a SMS reminder the day before the start of his “week”.

### *Case inclusion criteria*

Cases were eligible if they meet the above case definition and accepted to participate. An oral and/or written informed consent was requested to ILI cases after explaining the objectives of the study.

### *Case exclusion criteria*

Cases were excluded if they:

- refused to participate in the study;
- were not eligible for influenza vaccination;
- were institutionalised;
- were unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons.

All the excluded cases were registered in an appropriated form.

## **Control groups**

### *ILI influenza negative controls*

Considering the test negative design, **Controls**, corresponded to individuals that presented ILI symptoms to a participating GP but were laboratory tested negative for influenza infection -

A(H1N1), A(H3N2), B and A(H1N1)2009 virus.

As for **Cases**, **Controls** were systematically selected from the GP list or other, provided that an encounter patient/GP took place. The systematic sampling procedure was already described (for **Cases**).

The exclusion criteria described for **Cases** are also applicable for **Controls**. Excluded controls were registered in an appropriated form.

### *Community controls*

This group of controls was selected from an already implemented vaccine coverage monitoring survey, conducted every year since 1998<sup>7</sup>.

Controls were selected from a population-based dual-frame sample of households with landline or mobile telephone. Data was collected via Computer Assisted Telephone Interview during March 2011. Information with interest for the current study comprised the vaccine status, influenza-like illness symptoms manifested from September to the interview date (yes or no answer) and presence of chronic conditions.

## **Exposure (vaccination)**

### *Target groups, vaccines in use*

The target groups for vaccination were all individuals belonging to a risk group (see below).

During the 2010-2011 influenza season, seasonal vaccines were available at pharmacies and several brands were in use, namely:

- Chiroflu, Novartis Vaccines and Diagnostics
- Fluad, Novartis Vaccines and Diagnostics
- Fluarix GlaxoSmithKline
- Inflexal, Berna Biotech Italia
- Influvac, Solvay Farma
- Istivac, Sanofi Pasteur MSD
- Istivac Infantil, Sanofi Pasteur MSD

Also available in Health Centres from National Health Service was the monovalent vaccine against the A(H1N1)2009 virus. Only one vaccine was in use, Pandemrix from GlaxoSmithKline.

## *Vaccination campaign*

Seasonal vaccination campaign started on week 38 of 2010 (September 2010).

### *Definition of vaccinated individual*

Seasonal vaccinated individuals:

- Individuals that had taken the seasonal vaccine (one of the available brands) 14 days before the disease onset;

Pandemic vaccinated individuals <sup>8</sup>:

- Full vaccinated – individuals with less than 10 years or immunocompromised that took the second dose of vaccine 14 days prior the onset of symptoms and all other patients with one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms;
- Full or partial vaccinated – individuals that has received at least one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms.

### *Vaccine status ascertainment*

Inoculation with 2010/2011 WHO approved influenza vaccine has been ascertained by the GPs by consulting the patient record and confirming explicitly with the patient if the vaccine was taken.

If no data existed in the clinical record, patients were asked about vaccine inoculation status. Flu patients have been asked if the inoculation was through a “shot”. The day and month of inoculation have been recorded and/or asked.

## **Risk groups**

Individuals were considered to belong to a risk group if in the GP records include or if the patient reports suffering from one of the underlying conditions included in the interview questionnaire.

Risk groups<sup>9</sup> were all patients with at least one of the following underlying conditions:

1. Diabetes: if treated for insulin or non-insulin-dependent diabetes;
2. Cardiovascular disease (myocardial infarction, angioplasty, coronary artery bypass

surgery, stroke, transient ischemic attacks, treated hypercholesterolemia, treated hypertension, treated hypercholesterolemia);

3. Chronic cardiac failure;
4. Chronic renal disease (chronic renal failure and nephrotic syndrome);
5. Chronic hepatic disease (cirrhosis, biliar atresia and chronic hepatitis)
6. Chronic respiratory disease (asthma, chronic bronchitis, emphysema, brochopulmonary dysplasia, cystic fibrosis, pneumoconiosis and pulmonary fibrosis)
7. Immunodeficiency (conditions that suppress the immune function due to underlying disease and/or therapy, e.g. chemotherapy, HIV infection);
8. Pregnant women in the second and third trimester.

For the pandemic vaccine only<sup>10</sup>,

1. Morbid obesity ( $\leq 10$  years and  $IMC \geq 25$ ;  $> 10$  and  $< 18$  years and  $IMC \geq 35$ ; adults  $\geq 18$  years and  $IMC \geq 40$ ).

### Confounding factors and effect modifiers

Data on confounding factors and effect modifiers were collected using a standardised questionnaire. For **Cases** and **Controls** selected at GP practices, data was collected on a face-to-face interview.

The questionnaire (in annex B) was elaborated in order to collect information on the risk groups plus the following variables:

- *Previous influenza vaccination (2009-2010)*: vaccination against seasonal and pandemic influenza in the last season (vaccination information for each vaccine);
- *Target group for vaccination*: patient belongs to the 2010-2011 seasonal vaccine target group, according to General Directorate for Health recommendations<sup>9</sup>;
- *Severity*: the severity of the underlying conditions was measured by the number of hospital admissions due to underlying conditions in the 12 months prior to inclusion in the study;
- *Smoking status*: smoking history was collected and coded as follows: never-smoker, former smoker (stopped smoking at least one year before inclusion in the study), current smoker;

- *Number of GP visits in previous year:* in order to document and control for health seeking behavior the number of all GP visits in the 12 months before inclusion in the study were recorded.
- *Co-habitants:* number of co-habitants was recorded considering the number of individuals that live with the patient (with or without family relations), excluding the patient;
- *Functional status:* low functional status was defined as needing help to bath.
- *Antiviral administration:* use of antivirals was documented when applicable. Type and date of administration was registered.

### Sample size calculation

The ILI cases sample size for the case control test negative design was set at 320.

This value was calculated in order to estimate a vaccine effectiveness of 70% with a lower bound of the 95% confidence interval equal to 35%, assuming that the seasonal vaccine coverage in controls was 20% (all age groups) and that the proportion ILI cases positive for influenza in the season would be 40%.

The expected vaccine effectiveness was set according to the estimates obtained in the 2009-2010 I-MOVE multicentre case control study, the assumed controls vaccine coverage was obtained from the 2009-2010 vaccine coverage telephone survey conducted in Portugal and the proportion of positives for influenza was the observed in the Portuguese sentinel surveillance system in the 2009-2010 season.

The sample size formula was adapted from<sup>11</sup>, that assumes a case control relation of 1:1 cases, in order to account for a given proportion of ILI cases positive for influenza, i.e. the case to control relation.

The sample size of ILI cases n will be given by:

$$\frac{z_{1-\alpha/2}^2 \cdot p_2 \cdot p_1 \cdot \epsilon}{VE}$$

Were,  $z_{1-\alpha/2}$  is the standard Normal distribution  $1-\alpha/2$  percentile,  $p_2$  is the vaccination coverage in the controls,  $p_1$  is the proportion ILI cases positive for influenza,  $\epsilon$  is relative precision and  $VE$  is the expected vaccine effectiveness.

During the 2009-2010 study 244 ILI cases were recruited by 32 GP's, that represents an

average of approximately 8 ILI cases per GP. This average was obtained even when the study period (week 50 to week 14) covered only 3 weeks of the epidemic period.

In this context we consider that for the 2010-2011 study if this average of ILI cases per GP is observed the sample of 60 GP's would be sufficient to achieve the needed 320 ILI cases.

In synthesis our goal will be to enroll 60 GP's to collect during all the epidemic period 320 ILI cases, to obtain 130 influenza positive cases and 190 ILI test negative controls.

The control group 2 (community controls) sample size is approximately 1000 household representing 3000 individuals from all ages.

## Data

### *Data collection for cases and controls*

Data on **Cases** and **Controls** were collected at GP office level. GPs interviewed the patients using a standardized questionnaire (in annex B). Each participating GP filled in the **Case** or **Control** questionnaire that included data on:

1. Demographics;
2. Signs, symptoms, date of onset of ILI;
3. Laboratory results;
4. Antiviral administration;
5. Current season influenza vaccination;
6. Previous influenza seasonal and pandemic vaccination (2009-2010);
7. Target group for vaccination;
8. Pregnancy;
9. Morbid obesity;
10. Smoking status;
11. Selected underlying chronic conditions;
12. Number of hospitalizations in the last 12 months;
13. Number of GP consultations in the last 12 months;
14. Number of completed years of education;
15. Number of co-habitants;
16. Functional status

### *Transmission*

On a daily basis, biological material (from the swab collection) and data from ILI cases were

sent by mail to the Instituto Nacional de Saúde Dr. Ricardo Jorge where it was centrally treated. Laboratory results obtained by the Department of Infectious Diseases team were sent to the Department of Epidemiology team with ILI case code and influenza test results on a weekly basis.

In order to perform the pooled analysis of the data gathered by all the participating countries, data was also transmitted to Epiconcept. This transmission involved the data anonymization and codification according to the list of variables, definitions and coding previously provided to EpiConcept.

### *Entry*

Final data entry was performed at Department of Epidemiology of the Instituto Nacional de Saúde Dr. Ricardo Jorge on a STATA SE 11 database by typing in the answers from the questionnaires and laboratory results.

### *Validation*

Before data entry, a visual verification of missing and inconsistent values was done by the research team. After data entry a validation script was also run on the database.

Validation procedures included verification of the presence of impossible values and of possible inconsistencies in variables and between variables. All missing or inconsistent values were clarified with the corresponding GP.

Finally double data entry was performed. Values found incongruent were checked in paper questionnaires or by direct phone call to the GP and corrected in the final database.

### *Data cleaning*

All ILI cases that did not meet the EU ILI criteria were excluded from analysis. Comparison between the values from the paper questionnaires and the data entered on the database was performed.

When inconsistencies were found, the corresponding GP was contacted in order to clarify the data.

## **Analysis**

### *Coding and categorization of variables*

All categorical variables were previously coded<sup>12</sup> with exception to:

- the age group was created from the variable age and categorized in four classes: 0-4; 5-14; 15-64 and  $\geq 65$  years of age;
- the indicator variable of the delay between the onset of disease and swab less than 3 days data was computed from the number of days between the onset and the swab;
- the smoking status variable was recoded as 1- current smoker and 0- former and never smoker;
- the variables diabetes, cardiovascular disease, chronic cardiac failure, chronic renal disease, chronic hepatic disease, chronic respiratory disease and immunodeficiency were recoded into 1- any chronic disease (at least one of the previous list) and 0 – no chronic diseases.

The variables treated as numerical (discrete or continuous) were age, days between the onset of the symptoms and swab, number of previous hospitalizations due to the underlying chronic diseases in the last 12 months, number of education years, number of co-habitants and number of GP consultations in the last 12 months.

#### Case variables:

Three case definition variables were defined to estimate VE against all influenza, influenza A(H1N1)2009 and Influenza B:

- All influenza: 1- if ILI case was positive for any influenza virus; 0 – if ILI case was a Control (negative for all influenza virus);
- Influenza A(H1N1)2009: 1- if ILI case was positive for influenza virus A(H1N1)2009; 0 – if ILI case was a Control (negative for all influenza virus)
- Influenza B: 1- if ILI case was positive for influenza virus B; 0 – if ILI case was a Control (negative for all influenza virus).

#### Exposure to seasonal influenza vaccine variable:

*Vaccinated (coded 1)*- ILI case has taken the seasonal vaccine 14 days before the disease onset; *Not vaccinated (coded 0)*-all others

#### Exposure to pandemic influenza vaccine (two variables)

##### 1) Full exposure variable

*Vaccinated (coded 1)* - ILI case with less than 10 years or immunocompromised that has taken second dose of vaccine 14 days prior the onset of symptoms and all others ILI cases with one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms; *Not*

*vaccinated (coded 0)-all others.*

### *Comparison of group's characteristics*

**Cases** (ILI positive – all, A(H1N1)2009 and B) and **Controls** (ILI negative) were compared according to the following variables: age, sex, pregnancy, morbid obesity, smoking status, diabetes, cardiovascular disease, heart failure, renal failure, chronic hepatic disease, immunodeficiency, any chronic condition, previous seasonal and pandemic vaccines (2009-2010), belong to target group, help for bathing, number of hospitalizations in the previous 12 months, years of education, patient belong to the GP list, number of GP consultation in the previous 12 months, number of co-habitants and the ILI symptoms.

The comparisons were performed considering that the samples were independent.

Association between variable **Case/Control** and the categorical variables was evaluated by the Chi-squared test. If at least one of the table cells presented expected frequencies lower than 5, the Chi-squared test was substitute by the Fisher's Exact test.

Comparisons of numerical variables between groups (**Case/Control**) were performed using the non parametric test of Mann-Whitney.

### *Measure of effect*

The vaccine effectiveness was computed as  $VE=1-OR$  (crude) and  $aVE=1-aOR$  (adjusted) where OR and aOR is respectively the crude and adjusted odds ratio of being vaccinated within **Cases** versus **Controls**.

Specifically VE estimates were obtained for the Cases definition: all influenza, influenza A(H1N1)2009 and influenza B.

For the crude estimate, the exact 95% confidence interval of VE (OR) was obtained by the method described in Sahai H and Khurshid<sup>13</sup>. The confidence interval for the aVE was computed by the respective method of adjustment (non conditional Logistic Regression).

Vaccine effectiveness (crude and adjusted by age group and presence of at least one chronic disease) was also computed by comparing the proportion of vaccinated **Cases** with the vaccine coverage estimated on the Community control group using the screening method as described Farrington 1993<sup>14</sup>.

### *Stratified analysis*

Due to small sample size stratified analysis was not executed in the test-negative design study.

For the screening method, 2010-11 seasonal VE was estimated for: the age groups <65 and ≥65 years of age and according to the presence of at least one chronic condition.

### *Multivariable analysis*

The odds ratio of being vaccinated within **Cases** versus **Controls** was adjusted for possible confounders. Adjustment was performed using the non conditional logistic regression.

Potential confounders were included in the model if they changed crude vaccine OR estimate in at least 10% after adjustment by the Mantel-Haenszel method<sup>13</sup>.

### *Restricted analysis*

For each outcome, all influenza, influenza A(H1N1)2009 and influenza B, case and controls were respectively restricted to the period from the first to the last ILI patient positive for each outcome.

### *Software used for data entry, statistical analysis.*

All the results were obtained using the package of statistical programs STATA/SE 11<sup>15</sup>.

## **Logistical aspects**

### *Consent*

Each GP had the responsibility of obtaining oral and written consent from ILI cases, after giving adequate information on the general study characteristics.

### *Ethical approval*

The study protocol was submitted and authorized by the Comissão Nacional de Protecção de Dados (National Committee for Data Protection) and submitted to Comissão de Ética (Ethics Committee) of Instituto Nacional de Saúde Dr Ricardo Jorge, I.P (annex A).

### *Team*

*Department of Epidemiology, Instituto Nacional de Saúde Dr. Ricardo Jorge*

Ausenda Machado, Baltazar Nunes (coordinator), Inês Batista

*Department of Infectious Diseases, Instituto Nacional de Saúde Dr. Ricardo Jorge*

Patrícia Conde, Paulo Gonçalves, Pedro Pechirra (co-coordinator), Raquel Guiomar

## *General Directorate of Health*

Isabel Falcão

## *MS network and others GP*

(see Acknowledgments' section)

## *Supervision*

A supervising committee was established with participating members of the Direcção-Geral da Saúde (Directorate General of Health), INFARMED (National Authority of Medicines and Health Products), CEFAR/ANF (National Pharmacies Association) and APMCG (Portuguese Association of General Practitioners).

## *Training*

After the selection procedure, to each one of the GPs that agreed to participate, a personal interview was made by phone explaining the study and their participation. They also received the protocol, case definition questionnaires and laboratory swabbing procedures.

To all, has been described:

- the design of the project;
- the EU case definition;
- the inclusion and exclusion criteria to select ILI cases and underlined that selection should be independent of vaccination status;
- the definitions and concepts associated to each variable in the questionnaires and the way of answer or coding questions;
- to collect nasopharyngeal samples, and provide transportation to the National Influenza Reference laboratory in INSA;
- to accept data quality checks on the quality of some selected issues.

For these purposes several telephone calls have been made during the recruitment and development of the study. When necessary, some personal contacts or by e-mail have been made to clarify doubts.

## Results

### Participating GP's:

After the selection procedure, 58 GPs agreed to participate. About 60% (35) participated in the study by selecting, collecting swabs and data on **Cases** and **Controls**.

As mentioned, the total number of GPs accepting to participate in the study was 58, 23 were current active members of the MS network, whilst 35 GPs were ex-members of the network or others (Table 1).

**Table 1:** Number of GPs that accepted to participate and those that selected at least one ILI patient.

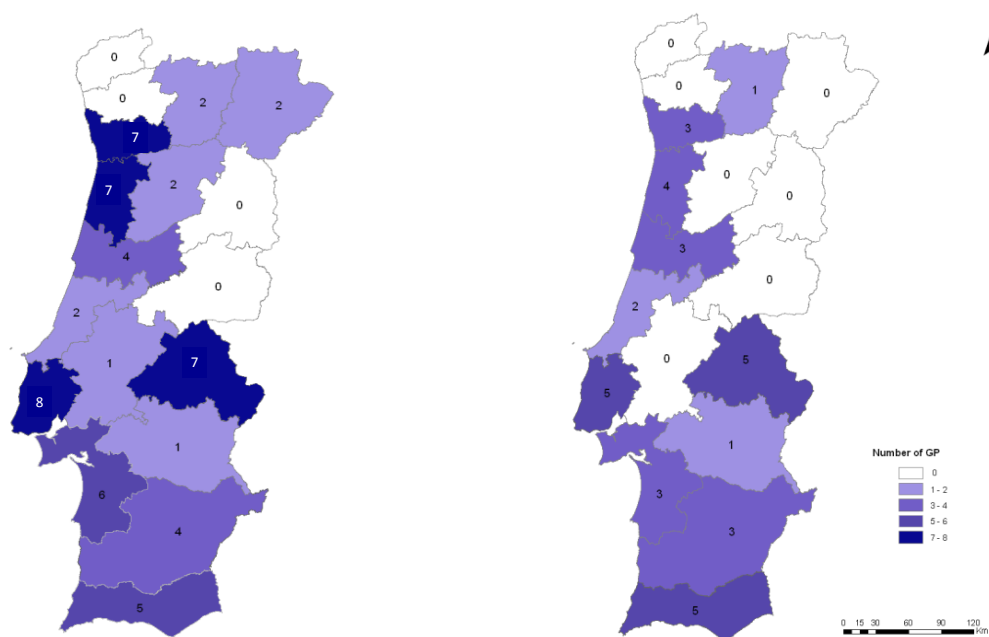
	GPs currently participating in MS <sup>1</sup>	GPs ex-participants in MS or others <sup>1</sup>	Total
All GPs accepting to participate	23	35	58
GPs reporting valid data	14	21	35

<sup>1</sup>MS – “Médicos-Sentinela” network

All participating GPs work in a Health Center of National Health Service (Ministry of Health) and have a stable list of patients. GPs that accepted to participate in Euroeva were distributed by all 5 Administrative Regions and by 14 of the 18 Districts of mainland Portugal. GPs reporting ILI cases covered all 5 regions and 12 of the Districts (Figure 1).

a)

b)



**Figure 1:** Distribution of participating a) and effectively reporting b) GPs.

Regarding the population covered by the Euroeva project, obtained by the sum of all the patients belonging to GP lists, it can be seen in Table 2 that small differences were found between the Euroeva age group distribution and the one observed in all MS network and the 2009 Portuguese population estimates. In detail, between Euroeva study population and population estimates, a difference (approximately 4%) is found in the extreme age groups, with 0-14 years age group underrepresented and the 65+ age group overrepresented.

**Table 2:** Distribution of the population covered by the Euroeva project and MS network compared with 2009 Portuguese population estimates.

	EUROEVA	MS network	Population 2009
0-14	11.2%	15.0%	15.2%
15-24	11.0%	10.9%	11.1%
25-64	59.3%	55.2%	55.8%
65+	22.6%	18.9%	17.9%

### Influenza season 2010-2011

Data collected through Portuguese surveillance system (Sentinel General Practitioners Network and Emergency Units Network) reveals that the influenza activity in Portugal, during 2010-11 season, was moderate and lower than the previous one.

As seen in Figure 2 the peak incidence rate was higher in 2009-10 season, but on the other hand, the epidemic period in 2010-11 lasted for 4 weeks more than 2009-10. The predominant virus circulating in 2010-11 was the A(H1N1)2009 (55.7%) which co-circulated with the B/Victoria strain (42.8%). This fact contrasted with 2009-10 season where almost all viruses detected were A(H1N1)2009 (93%).

#### *Virus circulation*

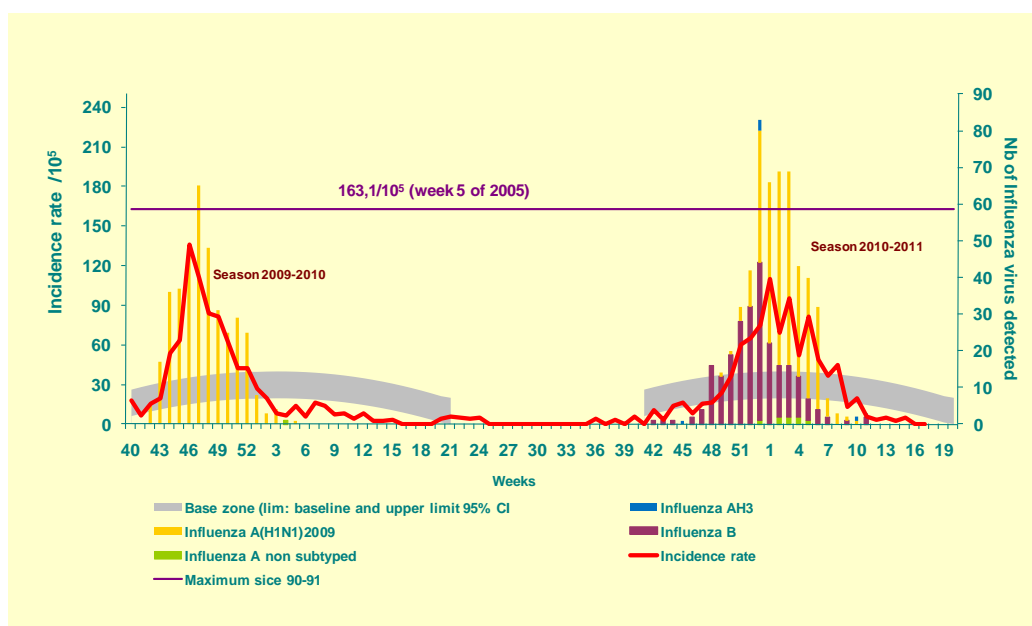
From October 2010 (week 41) to March 2011(week 11), the influenza B/Victoria virus has been in circulation (42.8% of cases). The pandemic influenza virus A(H1N1)2009, was detected in 55.7% of the cases, from December 2010 (week 48) to March 2011 (week 10), becoming the dominant subtype since week 1/2011. One can see that during most of the season, both A(H1N1)2009 and B/Victoria circulated simultaneously (Figure 2). Nevertheless, other subtypes were also identified in 1.5% of the cases: AH3, and B/Yamagata.

## Duration

The incidence rate increased, above the baseline threshold, from week 46/2010 to week 11/2011. By week 46 the flu incidence rate began to rise quickly, peaked at week 1/2011 with the value of  $109.7/10^5$  inhabitants, and began decreasing, by steps, till week 11, staying below the baseline until the end of the season.

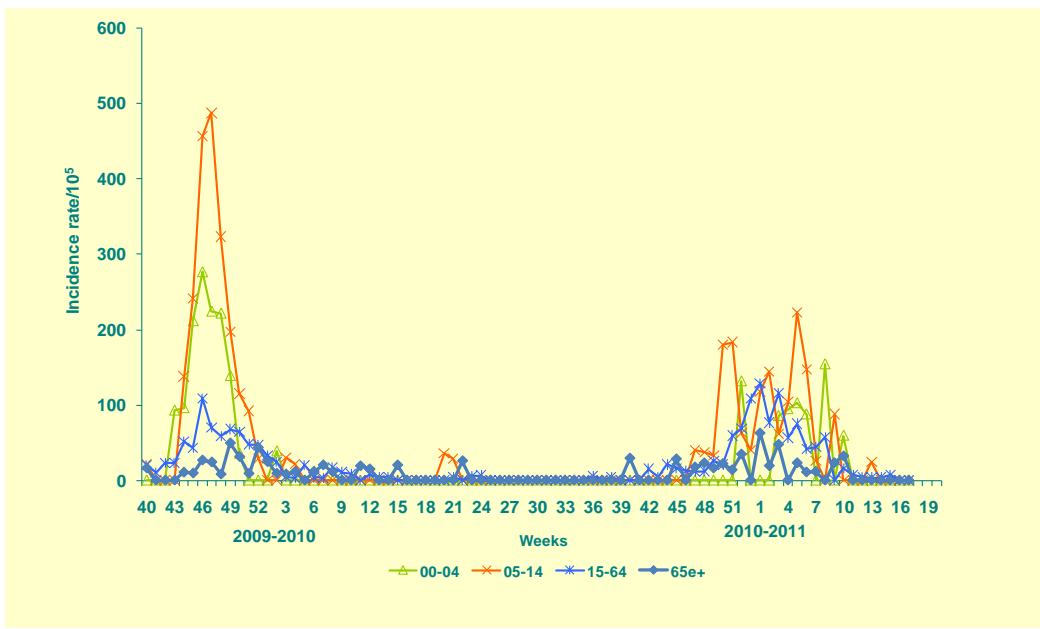
## ILI incidence

Comparing the epidemic period of 2010-2011 with the previous 2009-2010, one can see that incidence rate peaked earlier in 2009-2010 (week 46/2010) than in 2010-2011 (week 1/2011), respectively with the values of  $142.3/10^5$  and  $109.7/10^5$  inhabitants (Figure 2).



**Figure 2:** Distribution of provisional incidence rates and number of virus detected by week in 2009-2010 and 2010-2011 seasons.

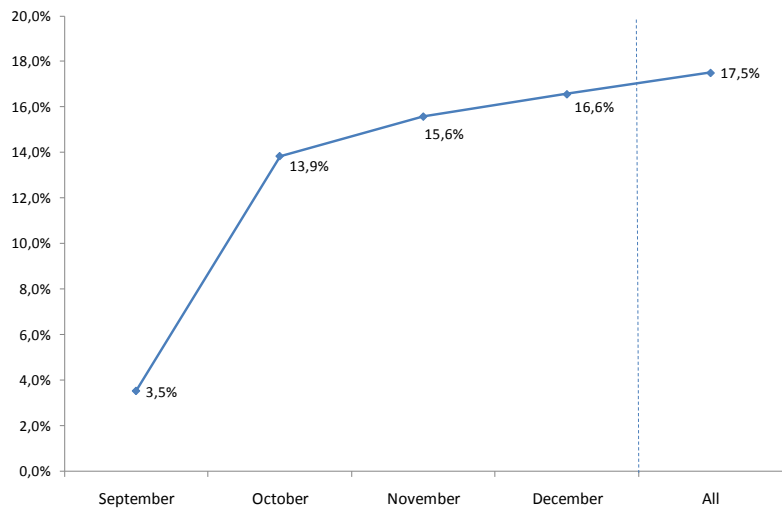
One can see that the higher incidence rates were estimated for the groups below 65 years, particularly for the group 05-14 years, on contrast to the seasons previous to pandemic. Nevertheless, when compared with 2009-2010 season one can verify that the youngest groups were the most affected by the disease, despite not so much as in the 2009-2010 season (Figure 3).



**Figure 3:** Distribution of provisional incidence rates by age group during 2009-2010 and 2010-2011 seasons.

*Vaccination*

In Portugal, the vaccination campaign started on week 38 of 2010. According to the results obtained with the yearly conducted dual-frame telephone survey<sup>16, 17</sup>, the vaccine coverage in 2010-2011 season was 17.5% (Figure 4).



**Figure 4:** Monthly evolution of the vaccine coverage (%) in the general population.

Considering the population target group for vaccination, the vaccine coverage for the elderly was 48.3% (CI95%: 40.9- 55.7) and 33.0% (95%CI: 28.4-37.9) for individuals with at least one chronic condition.

**Table 3:** Vaccine coverage (%) in the Portuguese population (**All**) and by age group and presence of a chronic condition.

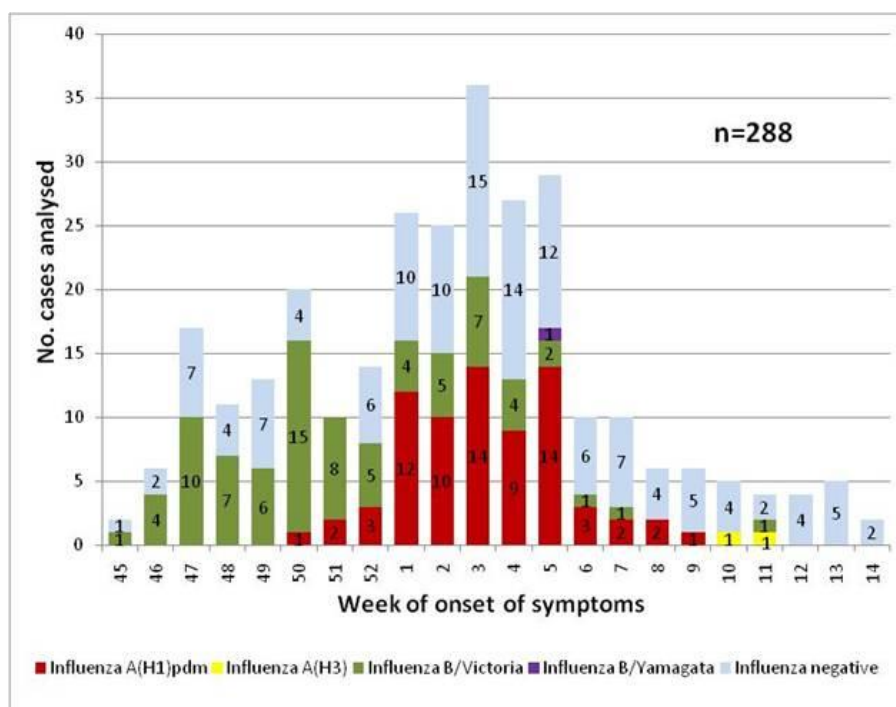
	<b>Vaccine Coverage,%,</b>	<b>(95% CI)</b>
All	17.5	(15.1-20.3)
0-64 yrs	10.8	(8.8-13.2)
≥65 yrs	48.3	(40.9-55.7)
No chronic	9.7	(7.6-12.3)
Any chronic	33.0	(28.4-37.9)

### Description of participants

During the study period, 290 ILI cases were selected by the participating GP’s, but four of them didn’t have or a biological sample for laboratory analysis (two cases) or a completed questionnaire (two cases). From the final 286 enrolled for the VE analysis, 33 were excluded (17 did not meet the EU ILI case definition, 5 did not meet the 7 maximum days between symptoms and swabbing and 11 were controls collected after disease onset week of last confirmed case). After the application of these restrictions 253 ILI cases were considered for the final data analysis.

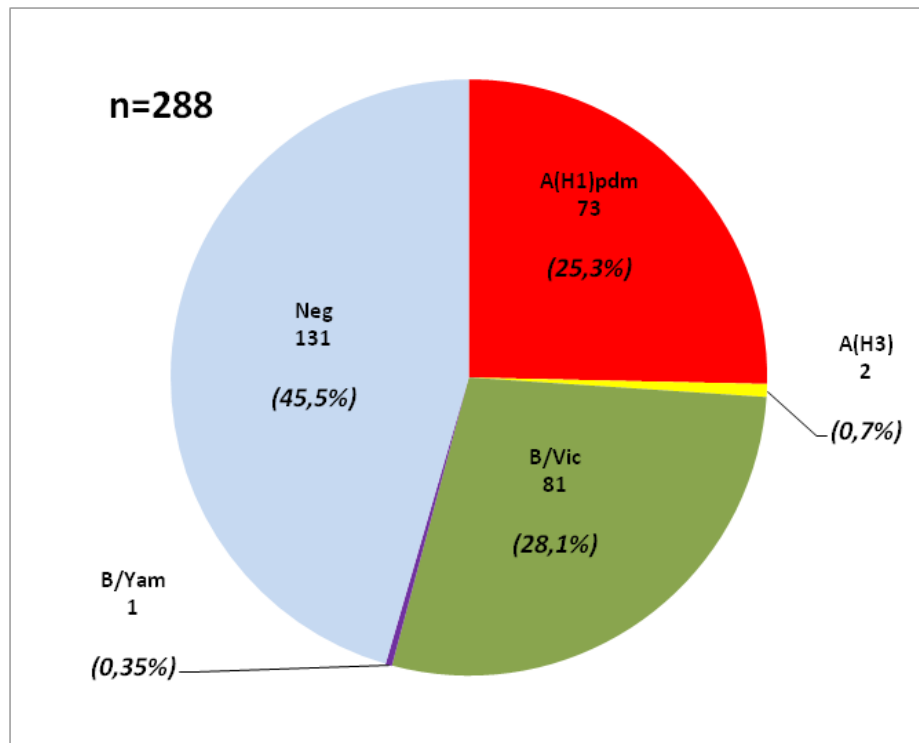
### Laboratory results

As referred previously, 288 specimens were collected, distributed in time as shown in Figure 5.



**Figure 5:** Weekly distribution of the 288 cases analyzed

Specimens were collected from week 45/2010 to 14/2011, being the majority of confirmed cases either influenza B/Victoria or pandemic influenza A(H1N1) viruses (Fig. 6). Sporadic influenza A (H3N2) and B/Yamagata viruses were also detected. Only 45.5% of collected specimens were negative for influenza virus.

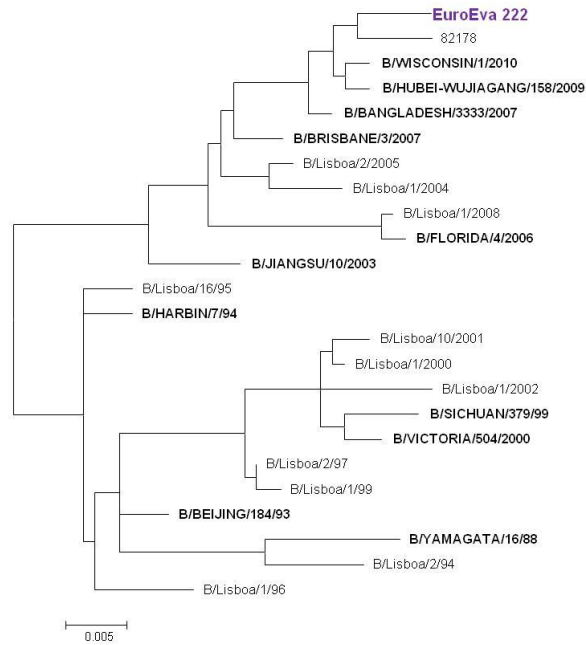


**Figure 6:** Characterization of ILI cases. The positive cases account for 54,5% from the total of collected biological products.

Influenza A viruses were not recovered from cell culture. Influenza B virus isolates were obtained from 32 specimens (20.5% of the ILI-positive cases). These were all antigenically similar to the vaccine strain B/Brisbane/60/2008.

Genetic analysis of the haemagglutinin gene was performed in 14 Influenza B/Victoria isolated strains (Fig.7). All analysed B/Victoria strains clustered into the phylogenetic clade of the vaccine strain B/Brisbane/60/2008, presenting the amino acid changes N75K, N165K and S172P, characteristic of this clade. Within this clade, 10 viral strains fell in the subgroup of B/Hong Kong/514/2009, carrying the L58P amino acid substitution.





**Figure 8:** Phylogenetic tree of influenza B/Yamagata HA1 subunit. The only one influenza B/Yamagata virus analysed in this study (EuroEva 222) is genetically closely related to the reference strain B/Wisconsin/1/2010. The HA sequence of the reference strains (in bold) were obtained from GISAID.

### *Description of Cases and Controls*

For analysis purpose, data was restricted to the 253 ILI cases obtained after exclusion criteria application. From these, 144 were positive for an influenza virus, and were considered as **Cases** (and will be referred as **All Influenza**) and 109 that tested negative for influenza were considered as **Controls**. Within **Cases**, 73 were positive for B virus (and will be referred as **Influenza B**); 69 were positive for A(H1N1)2009 virus (and will be referred as **Influenza A(H1N1)2009**) and 2 were A(H3N1) virus. Due to the small proportion of this last virus type, no further comparisons with controls were made.

The following significant differences were identified when comparing cases to controls:

1. Clinical symptoms (see Table 4 and 5):
  - a. All **Influenza B** presented fever (94.2% of the **Controls** had this symptom);
  - b. Cough was present in a higher percentage in **All influenza, Influenza B** and **Influenza A(H1N1)2009** when compared to **Controls** (respectively, 97.9%, 97.3% and 98.6% vs 87.9%);
  - c. **Controls** indicated in a higher percentage to feel sore throat (84.9% vs 67.6% for **Influenza A(H1N1)2009**);
2. **Cases** were younger than **Controls** (mean age: 28 for **All influenza**, 22 for **Influenza B** vs 40 for **Controls**);

3. Previous seasonal vaccine (2009-2010) was higher in **Controls** (15.9%) than in **All influenza** (6.3%);
4. **Controls** presented a higher prevalence of any chronic condition (30.3% vs 11.1 for **All influenza**, 9.6 for **Influenza B** and 13.0% for **Influenza A(H1N1)2009**);
5. **Controls** presented a higher percentage of patients that belonged to the vaccination target group (38.5% vs 14.6% in **All influenza**, 16.4% **Influenza B** and 13.0% **Influenza A(H1N1)2009**);
6. **Controls** had a higher median number of visits to the GP over the last 12 months (**Controls** 3, **Influenza A(H1N1)2009** 2 and **Influenza A(H1N1)2009** 2);
7. Cases presented a higher median number of co-habitants (**All influenza**:3, **Influenza B**: 3 vs **Controls**:2).

**Table 4:** Description of Cases (**All influenza, Influenza B, Influenza A(H1N1)2009**) and **Control**, week's 45-11 influenza season 2010-2011, by time between the onset and swab, the symptoms, signs and treatment with antiviral.

		<b>All influenza</b>	<b>Influenza B</b>	<b>Influenza A(H1N1)2009</b>	<b>Controls</b>
Time between onset and swab collection (hours)	<i>mean</i>	2.0	1.9	2.0	1.9
	<i>p<sup>a</sup></i>	0.285	0.351	0.393	
	<i>less than 72h, %</i>	87.5 (144)	90.4 (73)	85.5 (69)	87.2 (109)
	<i>p<sup>b</sup></i>	1.000	0.638	0.460	
Fever, %		97.9 (143)	100.0 (73)	95.6 (68)	94.2 (104)
	<i>p<sup>c</sup></i>	0.128	0.037	0.696	
Malaise, %		91.7 (144)	91.8 (73)	91.3 (69)	91.4 (105)
	<i>p<sup>b</sup></i>	1.000	1.000	1.000	
Headache, %		83.2 (143)	88.9 (72)	76.8 (69)	79.8 (99)
	<i>p<sup>b</sup></i>	0.612	0.145	0.703	
Myalgias, %		88.7 (141)	81.9 (72)	95.5 (67)	90.3 (103)
	<i>p<sup>c</sup></i>	0.491	0.064	0.297	
Cough, %		97.9 (144)	97.3 (73)	98.6 (69)	87.9 (107)
	<i>p<sup>b</sup></i>	0.003	0.029	0.010	
Sore throat, %		72.9 (140)	78.6 (70)	67.6 (68)	84.9 (106)
	<i>p<sup>b</sup></i>	0.066	0.436	0.026	
Shortness of breath, %		22.4 (143)	19.2 (73)	23.5 (68)	14.6 (103)
	<i>p<sup>b</sup></i>	0.071	0.301	0.100	
Antiviral use, %		0.7 (140)	0.0 (73)	1.4 (69)	0.0 (109)
	<i>p<sup>c</sup></i>	0.377	-	0.208	

( ), number of valid answers; <sup>a</sup> Mann-Whitney test; <sup>b</sup> Fisher's Exact test, <sup>c</sup> Chi-squared test; - Not computed

**Table 5:** Description of cases and control, week's 45-11 influenza season 2010-2011, by age, sex, pregnancy, morbid obesity, smokers, seasonal vaccine in previous season and pandemic vaccine in season 2010-2011 and in previous season.

	All influenza	Influenza B	Influenza A(H1N1)2009	Controls
Age, mean	28	22	34	40
<i>p</i> <sup>a</sup>	<0.001	<0.001	0.094	
0-4 years, %	3.5	2.7	4.3	7.3
5-14 years, %	22.9	37.0	8.7	7.3
15-64 years, %	70.8	57.5	84.1	67.0
≥65 years, %	2.8	2.7	2.9	18.3
<i>p</i> <sup>b</sup>	<0.001	<0.001	0.014	
<hr/>				
Sex, male %	41.0 (144)	46.6 (73)	36.2 (69)	45.0 (109)
<i>p</i> <sup>c</sup>	0.608	0.880	0.277	
Pregnant women's, %	1.7 (60)	4.0 (25)	0.0 (33)	5.9 (34)
<i>p</i> <sup>b</sup>	0.264	0.745	0.208	
Morbid obesity, %	1.4 (144)	1.4 (73)	1.4 (69)	0.9 (107)
<i>p</i> <sup>b</sup>	0.737	0.779	0.748	
Smokers, %	15.3 (144)	6.8 (73)	23.2 (69)	16.8 (107)
<i>p</i> <sup>b</sup>	0.740	0.091	0.509	
Pandemic vaccine 2010-11, %	0.0 (144)	0.0 (73)	0.0 (69)	0.9 (109)
<i>p</i> <sup>b</sup>	0.249	0.412	0.425	
Seasonal vaccine 2009-10, %	6.3 (144)	6.8 (73)	5.8 (69)	15.6 (109)
<i>p</i> <sup>c</sup>	0.021	0.104	0.057	
Pandemic vaccine 2009-10, %	8.3 (144)	11.0 (73)	5.8 (69)	10.1 (109)
<i>p</i> <sup>c</sup>	0.663	1.000	0.411	0.411

( ), number of valid answers; <sup>a</sup> Mann-Whitney test; <sup>b</sup> Chi-squared test; <sup>c</sup> Fisher's Exact test

**Table 6:** Description of cases and control, week's 45-11 influenza season 2010-2011, by chronic conditions (any), need help for bathing, belongs to GP patient list, belongs to target group for vaccination, GP consultations and hospitalizations in the last 12 months, years of education and number of co-habitants.

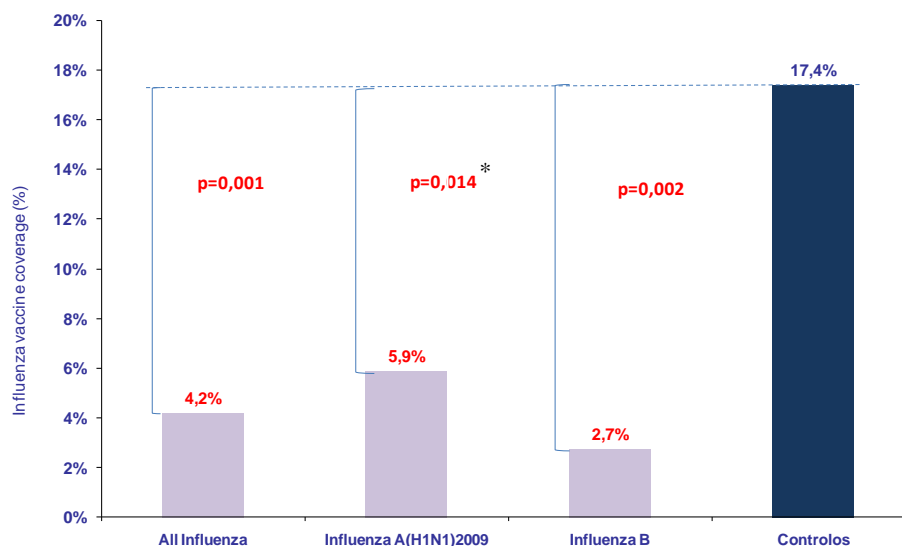
	<b>All influenza</b>	<b>Influenza B</b>	<b>Influenza A(H1N1)2009</b>	<b>Controls</b>
Chronic diseases (any), %	11.1 (144)	9.6 (73)	13.0 (69)	30.3 (109)
p <sup>a</sup>	<0.001	0.001	0.011	
Help for bathing, %	0.8 (124)	0.0 (58)	1.6 (64)	2.0 (98)
p <sup>b</sup>	0.429	0.274	0.825	
Belongs GP patient list, %	59.0 (144)	53.4 (73)	63.8 (69)	58.7 (109)
p <sup>a</sup>	1.000	0.542	0.532	
Vaccination target group, %	14.6 (144)	16.4 (73)	13.0 (68)	38.5 (109)
p <sup>a</sup>	<0.001	0.002	<0.001	
GP consultations last 12 mo, median	2.0	3.0	2.0	3.0
p <sup>c</sup>	0.016	0.121	0.010	
Hospitalizations, %	0.0 (142)	0 (73)	0 (69)	0.9 (109)
p <sup>b</sup>	0.253	0.412	0.425	
Years of education, median	8.0	8.0	9.0	9.0
p <sup>c</sup>	0.748	0.319	0.785	
Co-habitants, median	3	3	3	2
p <sup>c</sup>	0.003	0.001	0.162	

( ), number of valid answers; <sup>a</sup> Fisher's Exact test; <sup>b</sup> Chi-squared test; <sup>c</sup> Mann-Whitney test

## Vaccine coverage

### A) Test Negative Design

In this season, 2010-2011, the seasonal vaccine coverage in **Controls** was significantly higher (17.4%) than in **All Influenza** cases (4.2%), **Influenza B** (2.7%) and **Influenza A(H1N1)2009** (5.9%) (Figure 9).



\* comparison with a vaccine coverage of 19.3% in controls due to different time period restriction (week 50 –week 9).

**Figure 9:** Vaccine coverage of Cases - **All influenza, Influenza A(H1N1)2009** and **Influenza B-** and **Control** (test-negative design).

### B) Screening method

Considering the *Screening method* approach, and for **All individuals**, once again it was observed that the vaccine coverage in controls (from the community) was higher than the coverage in **ILI cases** and **ILI+** (Table 7).

However, after restringing the analysis to the target group population, the vaccine coverage in the **ILI+** with 65 years or more was slightly higher than the corresponding coverage in Controls. Nevertheless, this result should be carefully interpreted, since the coverage on **ILI+** was estimated based on a small number of cases (only 4).

**Table 7:** Description of Cases (**ILI** and **ILI** positive for an influenza virus- **ILI+**) and **Community control** (screening method), by vaccine coverage.

	Vaccine coverage (%)		Community control %, (95%CI)
	ILI (v/n)	ILI + (v/n)	
All	<b>9.7</b> (25/257)	<b>4.2</b> (6/143)	<b>17.5</b> (15.1-20.3)
0-64 yrs	<b>6.0</b> (14/232)	<b>2.9</b> (4/140)	<b>10.8</b> (8.8-13.2)
≥65 yrs	<b>44.0</b> (11/25)	<b>50.0</b> (2/4)	<b>48.3</b> (40.9-55.7)
No chronic	<b>4.4</b> (9/206)	<b>2.3</b> (3/128)	<b>9.7</b> (7.6-12.3)
Any chronic	<b>31.4</b> (16/51)	<b>18.8</b> (3/16)	<b>33.0</b> (28.4-37.9)

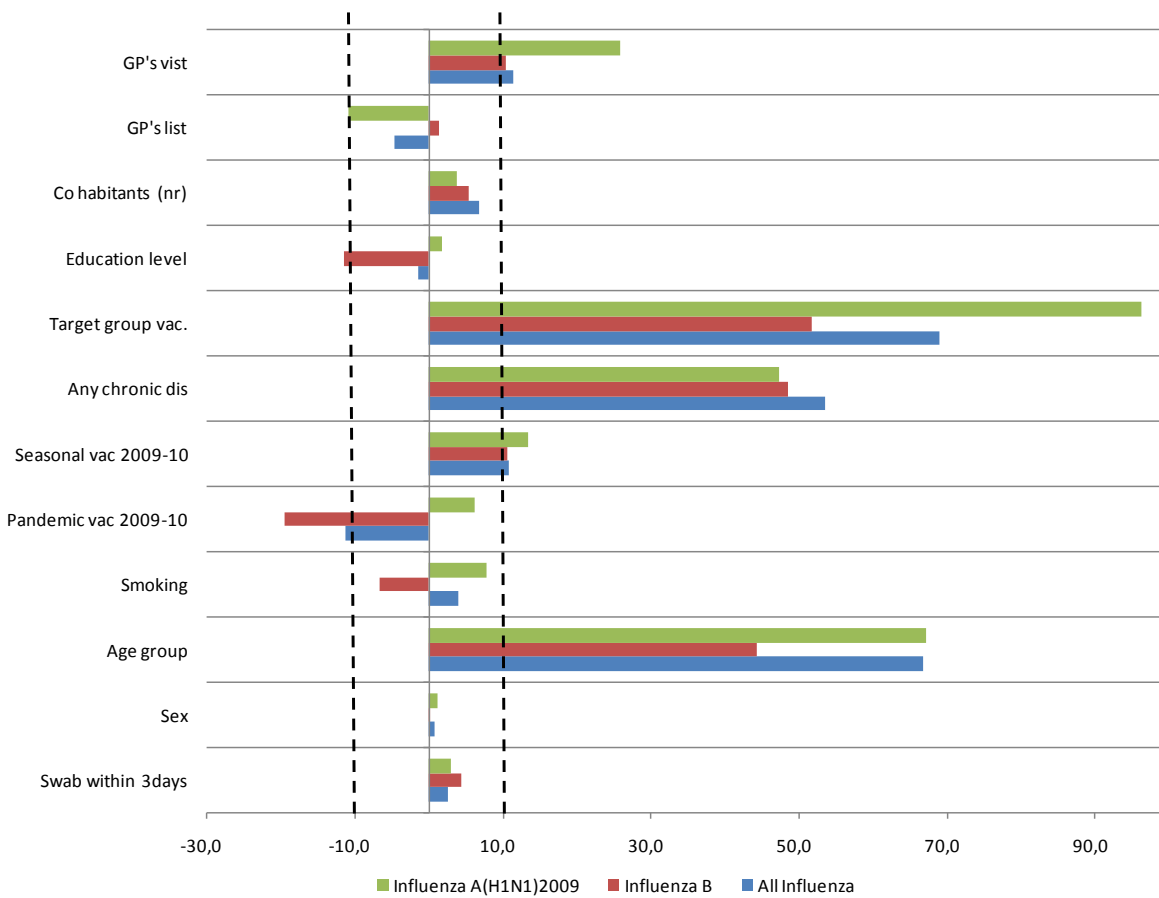
v – nr of vaccinated; n – nr of cases

## Vaccine effectiveness

### A) Test-negative design

According to the different outcomes in consideration, the crude point vaccine effectiveness estimates varied from 74.2% to 86.7%, all statistically significant (Table 8).

In order to estimate adjusted VE, it was adopted a strategy that consisted in including only potential confounders that changed 2010-11 seasonal vaccine crude OR more than 10% after M-H adjustment. In Figure 10 is presented the percentage that each potential confounder changed the 2010-11 seasonal vaccine crude OR after respective adjustment.



**Figure 10:** Seasonal vaccine 2010-11 OR change (%) after adjustment for potential confounding factors.

As showed by Figure10, factors that contribute more for changing the corresponding crude OR were, for all the outcomes, the age group, presence of at least one chronic condition and belonging to the target group for vaccination.

For Influenza B outcome were additional included in the model the education level and the pandemic vaccine status in 2009-10. For Influenza A(H1N1)2009 outcome, important confounders were also the seasonal vaccine uptake in 2009-10 season, belonging to the GP's list and the number of visits in the previous year. These results were then used so as to obtain adjusted VE (Table 8).

**Table 8:** Crude and adjusted seasonal 2010-11 vaccine effectiveness against All influenza, Influenza B and Influenza A(H1N1)2009, estimates based on comparison of laboratory-confirmed influenza case subjects and test-negative control subjects , influenza season 2010-11, Country Portugal.

	Crude analysis			Adjusted by logistic regression	
	Ca/Co	VE (%) [CI95%]		VE (%) [CI95%]	
All Influenza <sup>a</sup>	144/109	79.4	[43.4, 93.5]	58.2	[-60.7,89.1]
Influenza B <sup>b</sup>	73/109	86.7	[41.2,98.5]	75.1	[-98.3,96.9]
Influenza A(H1N1)2009 <sup>c</sup>	69/83	74.2	[13.7,94.0]	33.7	[-254.9,87.6]

Ca/Co: number of cases (Ca) /number of Controls (Co) considered in the analysis

<sup>a</sup> Data used in the estimates from week 45 till week 11; VE estimates adjusted for age group, pandemic and seasonal vaccine 2009-10, any chronic disease, target group and month of onset;

<sup>b</sup> Data used in the estimates from week 45 till week 11; VE estimates adjusted for age group, pandemic and seasonal vaccine 2009-10, any chronic disease, target group, educational level and month of onset;

<sup>c</sup> Data used in the estimates from week 50 till week 9; VE estimates adjusted for age group, seasonal vaccine 2009-10, any chronic disease, target group, number of visits to the GP in the previous year, belong to GP's patient list and month of onset.

After the adjustment for confounders, via non conditional logistic regression, the VE estimates varied from 33.7% to 75.1%. None of these estimates was statistical significant, all of them presenting a very low precision.

## B) Screening method

The screening method approach, crude and adjusted VE were estimated and the results are presented in Table 9.

Crude and adjusted point estimates were higher considering ILI positive for an influenza virus as the outcome measure.

After adjustment for age and presence of at least one chronic condition, VE estimates were 33.3% for the ILI as the outcome and 63.7% for ILI+ as the outcome.

**Table 9:** Crude and adjusted seasonal vaccine effectiveness against All ILI cases and ILI positive for an influenza virus (ILI+), estimates based on comparison of laboratory-confirmed influenza case subjects and community controls , influenza season 2010-11.

Outcome		Crude		Adjusted*	
		VE (%)	CI95%	VE (%)	CI95%
ILI	All	47.3	21.0-64.9	33.3	-3.1-56.8
	0-64 yrs	43.1	4.0-66.3	41.7	-0.4-66.1
	65 + yrs	**		**	
	No chronic	57.5	17.1-78.2	56.2	14.1-77.7
	Any chronic	**		**	
ILI +	All	70.1	31.7-86.9	63.7	16.5-84.2
	0-64 yrs	87.2	65.3-95.2	74.0	18.2-91.7
	65 + yrs	**		**	
	No chronic	77.7	29.9-92.9	71.4	22.5-89.4
	Any chronic	**		**	

\* Adjusted for confounding (age group and presence of chronic diseases) using the Farrington method

\*\* Not computed due to small sample size

## Discussion

### Overall results

#### *Laboratory confirmed influenza outcome*

After adjustment for potential confounders the 2010-11 seasonal vaccine effectiveness against medical attended laboratory confirmed influenza was 58% (CI95% -61 – 89) using the case control Test-Negative Design (TND). For the same study design, but considering strain specific outcomes, the adjusted 2010-11 seasonal VE was 75% (CI95% -98 – 97) and 34% (CI95% -255 – 88) respectively for influenza B and influenza A(H1N1)2009. None of these estimates was statistical significant.

On the other hand adjusted (age group and any chronic disease) VE estimate for laboratory confirmed influenza (all types) using the screening method approach was 64% (CI95% 17 – 84).

Considering as outcome any influenza virus, VE point estimates obtained by the two methods were very similar (58% for TND and 64% screening method). One possible explanation for this consistency could be the very close 2010-11 seasonal vaccine coverage estimates between ILI laboratory influenza negative controls (17.4%) and the community controls sample ECOS (17.5%).

Another important observation is the fact that the VE estimated in this report are all in between the range, of the up to now, 2010-11 seasonal VE published estimates<sup>18-21</sup>, i.e., 42% to 65%. This result enhances the uniformity of the VE estimates and also indicating that according to the published results 2010-11 VE ranged between 42-65%.

#### *Influenza-like illness outcome*

The 2010-11 adjusted (age group and any chronic disease) seasonal VE against medically attended ILI was 33% (CI95% -3.1-56.8), result obtained only for the screening method. Comparing with the previous result obtained using laboratory confirmed influenza as outcome, VE is lower. This result was somewhat expected since, ILI outcome is a less specific measure than laboratory confirmed influenza.

Looking at the results published, up to now, the only 2010-11 seasonal VE estimate against medically attended ILI was obtained with a cohort of individuals with major chronic conditions in Navarra<sup>18</sup>, which obtained the VE of 31% (CI95% 20-40). Although the study population in this

work was different (only major chronic disease), our estimate for all population (including major chronic disease population) was very close (33% vs 31%).

### **Participation rate and representativeness of the covered population**

Overall 58 GP's agreed to participate in Euroeva 2010-11, from these 35 GP's contributed with ILI cases, which represents a 60% participation rate. Comparing to the 2009-2010 VE study, similar GP's participation rate was obtained (60%), even though in this season a reinforcement efforts of GP's participation with a monthly newsletter (4 issued) and the weekly data validation (by mobile phone) was done.

The number of ILI cases selected for the study (290) was lower than the sample size (320) defined in the protocol. Nevertheless the stated objective of estimating a 95% confidence interval lower limit for the crude VE of at least 35% was achieved (CI95% 43-94). This was due to the fact that estimated crude VE point estimate (79%) was higher than the 70% used in protocol for the sample size determination.

Participating GPs were volunteers for Euroeva as they are for participating on the MS network. Therefore they do not represent the total group of GPs working in health centers, in Mainland Portugal.

Nevertheless the age distribution of population covered by Euroeva project was slightly different from the age distribution observed in the MS network population and with the national estimates for 2009 (Table 2), with Euroeva population being somewhat overrepresented in the 25-64 and 65+ age groups.

### **ILI cases selection**

As in the previous study, for identification of ILI cases, the 2010-11 Euroeva study used the EU ILI definition. From all the ILI cases enrolled with complete biological and clinical information, only 17 did not meet the EU ILI case definition, corresponding to a 94% correct ILI case selection. This result represents an improvement, although small, given that in the last study (2009-10) a 92% correct ILI case selection was observed.

Given its success, the systematic selection scheme of ILI cases used in 2009-10, of randomly attributing a different first day of week to each GP (from Monday to Thursday) to start ILI cases selection, was once again used in 2010-11. Keeping the restriction of two ILI cases aged less

than 65 years per week, but without any limit to the number of 65+ ILI cases selected, given the low proportion of ILI cases with 65+ year of age obtained in 2009-10. Beside this effort, the proportion of ILI cases with 65 or more years of age continue to be considerable low, precluding any VE estimate for this age group.

## Controls

In the 2010-11 study, two groups of controls were considered in order to estimate VE. The ILI influenza negative cases and the community sample obtained by an independent routine procedure used to estimate seasonal vaccine coverage in the Portuguese population<sup>7</sup>.

The ILI influenza negative controls were particularly interesting since they were obtained directly from the routine surveillance system, just adding a number of variables to be used mainly in stratified analysis, effect modification and confounding. Also of substantial interest is the community control group selected from the Portuguese general population directly from an independent routine source that is easily accessed since is a national routine system to estimate seasonal vaccine coverage.

In order to have consistency between the 2010-11 seasonal VE estimates, obtained from the two used methods, it would be very important that vaccine coverage reached an equivalent level between the two control groups. In this study our expectation was completely fulfilled given that the obtained vaccine coverage's were 17.4% and 17.5%, respectively for the test-negative control group and community ECOS sample. These very close values resulted in very similar seasonal VE estimates obtained from the two used methods (58% and 64%).

Although the results are consistent and close between methods, at this point some remarks should be state regarding the use of the screening method. First, the source population used is not the GP's catchment area population but a national sample of households with mobile and landline phone. This fact could have bias the VE estimates given that the controls were selected from a broader source population than cases. Second, the vaccine coverage used in the screening method was assumed as known, but it was also obtained with a population sample, so the variance of this estimate should be included in the 95% confidence interval VE estimate. This was not done because the Farrington method does not allow it. Further developments should be done in order to include this information in VE estimate.

## **Vaccination status**

Considering the test negative design, the vaccination status was ascertained with the same approach between cases and controls. Besides the data entered in the patient medical record, GPs were asked to confirm the inoculation of vaccine with the patient and register the brand name of the vaccine. Complementarily, the GPs should verify if the inoculation had been through a shot. It is improbable that GPs used different ascertainment criteria, especially between their own ILI cases and controls.

## **Information bias**

Data on variables used to characterize cases and controls were collected by direct interview conducted by the participating GP. As the questionnaires were similar for both cases and controls and GP's did not know the laboratory result of the enrolled ILI case it is unlikely that GPs collect data differently in cases and controls.

Regarding the screening method it must be stressed the fact that information on the vaccine uptake and presence of chronic condition was self-reported for respondents or given by *proxy* for the remaining household members. This could introduce bias since information on vaccination status differs from cases (collected and validated by the GP) and controls (self reported). In order to diminish bias, only controls that referred that inoculation was through a shot, were considered as vaccinated.

## **Bias associated to the sensitivity of the case definition used in EUROEVA**

As stated before, cases were selected according to EU ILI definition. This EU ILI criterion consists in very stringent combination of symptoms and during this season a high percentage of ILI cases positive for influenza was observed (56.9%).

Nevertheless, it should be taken into consideration that by using this kind of case definition some ILI cases with milder symptoms may be discarded in the selection and this could introduce bias in our VE estimate. For instance, if vaccination induces the occurrence of true cases of influenza with a smaller number and "mild" symptoms/signs, GPs would have a higher probability of failing to select such "mild", but true cases, for lab confirmation, meaning that the GP criteria would be less sensitive for the vaccinated true cases. This fact would underestimate vaccine coverage in cases and over estimate VE. Even though it's recognized the possibility of this bias, inside the EUROEVA data it is not possible to estimate the dimension of it.

## Effects of adjustment

VE point estimates after adjustment presented an important difference from the crude ones, approximately a 10 to 20% decrease. Confounders were included in the models only if they changed more than 10% the crude seasonal vaccine OR. With this kind of approach, it was possible to identify positive confounders, *i.e.*, factors that overestimate VE, as well as negative confounders (that do the opposite effect on VE). Thus, within the major positive confounders, it was found that age group, presence of any chronic disease and belonging to target group for vaccination had major effect on VE estimates. As negative confounder, only the pandemic vaccination in 2009-10 status was found to produce relevant effects on VE estimates.

The three major confounder's age group, presence of chronic conditions and belonging to the 2010-11 seasonal vaccine target groups are high correlated factors. This situation could influence VE adjustment efficiency due to the presence of co linearity. Unfortunately, the small sample size precludes additional data exploration and a solid interpretation of the effects of adjustment.

## Conclusions

Overall results obtained by the Euroeva study indicate that crude 2010-11 seasonal VE estimate against medically attended influenza was 79% (CI95% 43-94) and 70% (CI95% 32-87), respectively for the TND and screening method. After adjustment the respective VE estimates decreased: 58 (CI95% -61-89) and 64% (CI95% 17-84). These last results were in accordance to the up to now published results (42-65%).

The TND study was also able to provide strain specific 2010-11 seasonal VE estimates: influenza B, crude VE=87% (CI95% 41-99) and adjusted VE=75% (CI95% -98-97) and for influenza A(H1N1)2009, crude VE=74% (CI95% 14-94) and adjusted VE=34% (CI95% -254-88).

These results suggest that the 2010-11 seasonal VE was lower than the monovalent A(H1N1)2009 VE estimated by the IMOVE multicenter study in the season 2009-10: 72% (CI95% 46-86).

Laboratory results also showed that all B/victoria viruses identified were antigenic and genetically similar to the vaccine strain B/Brisbane/60/2008.

Our study was unable to estimate VE for specific seasonal vaccine target groups. Although the efforts made to oversample elderly individuals ( $\geq 65$ ), the sample obtained continue to be insufficient.

This result enhances, as in previous studies, the unavoidable need for pooling data from a network of VE studies with common protocol as IMOVE.

## Recommendation

The main recommendations focused on:

- To calculate sample size taking into consideration:
  - the context of the multicentre case-control study: minimum sample size per site in order to assure a minimum homogeneity for pooled analysis;
  - different expected VE point estimates, i.e. for low, medium and high VE;
  - minimum set of factors for stratified analysis;
  - the adjustment for confounders.
- To increase sample size, mainly in the elderly population (aged 65 years or more);
- To increase the total number of participating GP's in the study by exploring other sources of GP's recruitment;
- To study the inclusion of the population based vaccine coverage uncertainty in the screening method;
- To explore with participating GP's the best way to obtain estimates of the Euroeva ILLI sample fraction.

Finally we also recommend continuing the harmonization of the study designs between participating countries with the multi-centre study objective.

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## **Annexes**

**Annex A -Project submission to the Ethics committee of the Instituto Nacional de Saúde Dr. Ricardo Jorge**

Submissão de estudo / projecto de investigação à CE

N.º de entrada na CE: \_\_\_\_ / \_\_\_\_

De: Baltazar Nunes

Data: 2011-05-31

Para: **Presidente da Comissão de Ética para a Saúde do INSA, I.P.**

C/C:

Assunto: **Pedido de apreciação e parecer – projecto EUROEVA 2010: Efectividade da Vacina Antigripal na Europa, na época 2010-2011**

Pretendendo realizar no Departamento de Epidemiologia do INSA, I.P., o projecto de investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador Principal, a sua apreciação e a elaboração do respectivo parecer.

Para o efeito, anexo toda a documentação referida na instrução de trabalho dessa Comissão respeitante a projectos de investigação.

Com os melhores cumprimentos,

Lisboa, 31 de Maio de 2011

O Investigador Principal

**Tomei conhecimento**

Concordo  Não concordo

Comentários

Coordenador/Responsável (Dep/Gab/Serv)

**Annex A2 -Project submission to the National Committee for Data Protection**

Processo n.º 6533/2010

**AUTORIZAÇÃO N.º 3142/2010**

O Instituto Nacional de Saúde Dr. Ricardo Jorge notificou à CNPD um tratamento de dados pessoais com a finalidade de elaborar um estudo observacional para determinar a efectividade da vacina antigripal na época 2010/2011 na população em geral e no grupo de indivíduos com idade igual ou superior a 65 anos (Projecto EUROEVA).

Serão incluídos no estudo os indivíduos que sejam vacinados contra a gripe, que preencham os critérios de inclusão e que se dirijam a qualquer um dos centros participantes. O médico assistente, investigador no estudo, solicitará consentimento informado, cuja declaração deverá ser arquivada no processo clínico do doente.

Os dados serão recolhidos num caderno de recolha de dados em formato papel.

No "caderno de recolha de dados" não há identificação nominal dos titulares, sendo aposto um código de doente. A chave desta codificação só pode ser conhecida do médico assistente.

Será recolhida uma amostra do exudado nasofaríngeo, que será analisada no Instituto Nacional de Saúde Dr. Ricardo Jorge. Não será constituído um biobanco.

Os destinatários deverão ser ainda informados sobre a natureza facultativa da sua participação e garantida confidencialidade no tratamento.

A CNPD já se pronunciou na sua Deliberação n.º 227 /2007 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios orientadores para o correcto cumprimento da Lei de Protecção de Dados, bem como as condições gerais aplicáveis ao tratamento de dados pessoais para esta finalidade.

No caso em apreço, a notificação enquadra-se no âmbito tipificado por aquela Deliberação.

A informação tratada é recolhida de forma lícita (art.º 5º, n.º1 al. a) da Lei 67/98), para finalidades determinadas, explícitas e legítimas (cf. al. b) do mesmo artigo) e não é excessiva.

O fundamento de legitimidade é o consentimento expresso do titular dos dados.



COMISSÃO NACIONAL  
DE PROTECÇÃO DE DADOS

Assim, nos termos do nº2 do artigo 7º e da alínea a) do nº 1 do artigo 28º da LPD, com as condições e limites fixados na referida Deliberação, que se dão aqui por reproduzidos e que fundamentam esta decisão, e ainda com a condição aqui fixada, autoriza-se o acesso aos dados pessoais dos doentes, constantes dos processos clínicos, para a elaboração do presente estudo.

**Responsável pelo tratamento:** Instituto Nacional de Saúde Dr. Ricardo Jorge

**Finalidade:** estudo observacional para determinar a efectividade da vacina antigripal na época 2010/2011 na população em geral e no grupo de indivíduos com idade igual ou superior a 65 anos (Projecto EUROEVA).

**Categoria de Dados pessoais tratados:** código do doente, dados demográficos (sexo e idade), sinais e sintomas de síndrome gripal, resultados laboratoriais do diagnóstico de gripe, vacinação antigripal, doenças crónicas, gravidez, hospitalizações nos últimos 12 meses, nº de consultas de medicina geral e familiar nos últimos 12 meses, nº de anos de escolaridade, nº de co-habitantes na unidade de alojamento, necessidade de assistência no banho e hábitos tabágicos.

**Entidades a quem podem ser comunicados:** Não há.

**Formas de exercício do direito de acesso e rectificação:** junto do médico assistente.

**Interconexões de tratamentos:** Não há.


**Transferências de dados para países terceiros:** Não há

**Prazo de conservação:** o código do titular deve ser destruído um mês após o fim do estudo.

**Dos termos e condições fixados na Deliberação n.º 227/ 2007 e na presente Autorização decorrem obrigações que o responsável deve cumprir. Deve, igualmente, dar conhecimento dessas condições a todos os intervenientes no circuito de informação.**

Lisboa, 13 de Setembro de 2010

Ana Roque, Luís Paiva de Andrade (Relator), Vasco Almeida, Helena Delgado António, Carlos Campos Lobo, Luís Barroso

  
Luís Lingnau da Silveira (Presidente)

# DECLARAÇÃO DE CONSENTIMENTO

## Projecto EUROEVA – Estudo da Efectividade da Vacina Antigripal

Fui informado(a) sobre os objectivos gerais do estudo e compreendi com clareza o que me é pedido como participante.

Fui ainda informado que:

1. Os dados a fornecer, bem como os resultados das análises que forem efectuadas sobre as amostras do meu exudado nasofaríngeo, serão estritamente confidenciais. Assim que os procedimentos do estudo o permitam esses dados e resultados serão tomados anónimos, isto é, deixarão de poder ser relacionados com a minha identificação;
2. Todos os investigadores e técnicos que utilize nesses dados estarão obrigados a segredo profissional;
3. Os resultados do estudo que venham a ser tomados públicos nunca incluirão o meu nome ou qualquer elemento que permita identificar-me.

Nestas condições, declaro que aceito participar no estudo, disponibilizando-me para:

1. Ser entrevistado e prestar informações sobre vários aspectos relacionados com a minha saúde bem como a algumas características pessoais relevantes;
2. Permitir a colheita de uma amostra de exudado nasofaríngeo para realizar várias análises relacionadas com este estudo ou com outros estudos que venham a ser realizados no futuro.

Data: \_\_\_\_ / \_\_\_\_\_ / 20\_\_\_\_

**Assinatura do participante:** \_\_\_\_\_

**Assinatura do médico assistente:** \_\_\_\_\_

**O(A) Investigador(a) responsável:**

Nome: Baltazar Nunes

Assinatura: \_\_\_\_\_

**Annex B – Questionnaires**

Nome:.....

N.º Cartão de utente/ Proc. Clínico..... Código do caso: 0101\_001

(Destacar por aqui)

Instituto Nacional de Saúde  
Doutor Ricardo Jorge



Código do caso: 0101\_001

## Efectividade da Vacina Antigripal EuroEva - 2010/2011

### Notificação Clínica da Síndrome Gripal

#### Colheita de Produto Biológico

Centro de Saúde..... Hospital..... Médico..... (pode ser substituído pela vinheta) Telefone..... Data da colheita....._/___/___ Hora da colheita ..... _ _	<b>A Preencher pelo INSA</b> N.º Lab: .....Nº Req.:.....  Data: ___/___/___ N.º Amostra.....  <u>Pesquisa de vírus Influenza</u> Resultado: .....
--	---

<b>Informação Relativa ao Doente</b> Sexo..... M F       Idade:.... _ _  anos.... _ _  meses
---

<b>Síndrome Gripal</b> Nova definição de caso, ECDC: A+1 sint.de B +1 sint.de C Data de início dos sintomas....._/___/___ A - Início súbito (<24h)..... S N D  B - Febre ou febrícula ___, ___ °C..... S N D  Mal-estar geral, debilidade, prostração..... S N D  Cefaleia..... S N D  Mialgias, dores generalizadas..... S N D  C - Tosse..... S N D  Dor de garganta, inflamação da mucosa nasal e faríngea, sem sinais respiratórios relevantes..... S N D  Dificuldade respiratória..... S N D  Calafrios/Arrepios..... S N D  Contacto com doente com gripe..... S N D	<b>Tomou antivirais durante os últimos 14 dias?</b> <input type="checkbox"/> Não <input type="checkbox"/> Sim, o doente <input type="checkbox"/> Sim, um co-habitante Nome do antiviral ..... Vai ser tratado com antiviral?  S N  Nome do antiviral .....
--	--

Legenda: M – Masculino F – Feminino S – Sim N – Não D - Desconhece

**Questionário Síndrome Gripal** (Confirmar as respostas directamente com o(a) doente)

EUROEVA

O doente não pertence à minha lista	__	Código do caso	0101_001			
<b>Data da consulta</b> em que este(a) doente foi seleccionado		____/____/____				
<b>Nesta época (2010/2011), o(a) doente foi <u>vacinado(a) contra a gripe?</u></b>		S	N	D		
<b>Se sim, qual foi a vacina usada, <u>trivalente (T) ou a monovalente A(H1N1)2009 (M)?</u></b>		T	M	D		
<b>Se sim, <u>vacinado em</u></b> (Se não sabe a data exacta, indique a mais aproximada)		____/____/____				
<b>Qual era o nome da vacina?</b>		_____ Não sabe  __				
<b>O(a) doente foi vacinado(a) contra a gripe sazonal na época 2009/2010?</b>		S	N	D		
<b>O(a) doente foi <u>vacinado(a) contra a gripe pandémica</u> na época 2009/2010?</b>		S	N	D		
<b>O(a) doente pertence ao grupo alvo prioritário para a vacina da gripe em 2010/2011?</b>		S	N	D		
<b>A doente encontra-se grávida?</b>		S	N	D	NA	
<b>Se sim em que trimestre se encontra?</b>		__	Não sabe  __			
<b>O (a) doente tem obesidade mórbida?</b> $\leq 10$ anos (IMC>25), >10 e <18 anos (IMC>35) e $\geq 18$ (IMC>40)		S	N	D		
<b>História tabágica do(a) doente</b> (Assinalar com X a opção relevante):						
O(a) doente fuma		__				
Deixou de fumar há mais de um ano		__				
Nunca fumou		__				
Não sabe se fuma		__				
<b>Doenças crónicas:</b>						
Diabetes		S	N	D		
Doenças cardiovasculares (acidente vascular cerebral, acidente isquémico transitório, enfarte de miocárdio, hipertensão arterial tratada, angioplastia, "by pass" coronário, hipercolesterolémia tratada)		S	N	D		
Insuficiência cardíaca crónica		S	N	D		
Doença renal crónica (falência renal crónica, síndrome nefrótico)		S	N	D		
Doença hepática crónica (cirrose, atresia biliar, hepatite crónica)		S	N	D		
Doença respiratória crónica (asma, bronquite crónica, enfisema, fibrose quística, pneumoconioses, displasia broncopulmonar, fibrose pulmonar)		S	N	D		
Imunodeficiência congénita ou adquirida		S	N	D		
Doença neuromuscular com compromisso da função respiratória		S	N	D		
<b>Nos últimos 12 meses, quantas vezes foi o(a) doente hospitalizado devido a uma destas doenças crónicas?</b>		__	Não sabe  __			
<b>Número de consultas de Medicina Geral e Familiar, nos últimos 12 meses.</b>		__	Não sabe  __			
<b>Quantos anos de escolaridade o(a) doente completou <u>com aproveitamento</u>?</b>		__	Não sabe  __			
<b>Quantas pessoas vivem na mesma casa com o(a) doente?</b> (familiares ou não familiares, sem contar com o doente)		__	Não sabe  __			
<b>O(a) doente necessita de ajuda para tomar banho?</b> (se tiver 10 ou mais anos de idade)		S	N	D	NA	

**Annex C – Instruction’s manual**



***EUROEVA***  
***"Efectividade da Vacina Antigripal"***  
***2010/2011***

Código:





## INSTRUÇÕES

O estudo visa estimar a efectividade da vacina contra a **gripe sazonal**, em indivíduos de **todas as idades** com particular enfoque em indivíduos com **idade igual ou superior a 65 anos**.

Este estudo tem um delineamento **caso-controlo** e terá início em **15 de Novembro de 2010**.

Gostaríamos que seleccionasse casos de síndrome gripal com **idade inferior a 65 anos** (2 casos por semana) bem como **TODOS** os casos de síndrome gripal com **65 e mais anos**.

A selecção de casos começa à **2ª feira**, com duração até ao final da época gripal, *i.e.*, semana 20 de 2011.

### AVALIAÇÃO DA EFECTIVIDADE DA VACINA DA GRIPE SAZONAL

#### MÉTODO

Pretende-se verificar se há diferenças na percentagem de vacinados, entre os **2** grupos seguintes:

1. casos de síndrome gripal com resultado laboratorial **positivo** para gripe;
2. casos de síndrome gripal com resultado laboratorial **negativo** para gripe.

Para a Vigilância da Síndrome Gripal, os exames laboratoriais a efectuar consistem no isolamento e na detecção do RNA do vírus da gripe. Para o isolamento do vírus e a detecção do RNA viral é necessário:

- Um **exsudado da nasofaringe** colhido durante os primeiros **5 dias** de evolução da doença (de preferência até ao 2º ou 3º dia) em zangaratoa cedida pelo INSA e enviada rapidamente pela Alfaloc, Transportes Expresso.

#### NOTA PARA MÉDICOS-SENTINELA

Se é **Médico-Sentinela** e já participa no programa de vigilância clínica e laboratorial da gripe, continue a fazê-lo como **habitualmente**. A única diferença é que, para este estudo, em **2 casos de síndrome gripal por semana com idade inferior a 65 anos e para TODOS os casos de síndrome gripal com 65 e mais anos**, terá de substituir a folha de preenchimento a que está habituado pela **folha do questionário**.

#### PROCEDIMENTOS

A cada médico participante será fornecido um caderno com **instruções**, **20 questionários**, **40 folhas para o consentimento informado (a serem preenchidas em duplicado por cada caso)**, e uma **folha para a recusa/exclusão** de casos para o estudo (**folha branca**). O questionário deverá ser preenchido sempre que identificar um caso de síndrome gripal na sua lista de utentes ou fora dela.

Note que os questionários estão pré-codificados com o **código de caso**, no canto superior direito, o que permite a respectiva identificação. Por favor registre, no cabeçalho do questionário, os dados de identificação pessoal de cada caso (Nome e N<sup>o</sup> SNS ou do Processo Clínico).

Cada médico receberá também um "kit" para colheita de exsudado **nasofaríngeo que deverá também ser identificado com o código de caso** (o mesmo que se encontra no canto superior direito do questionário).

## 1. Selecção dos casos de síndrome gripal

Selecione, na sua lista de utentes, ou fora dela, doentes com síndrome gripal. Deve identificar **2 casos de síndrome gripal com idade inferior a 65 anos e TODOS os casos de síndrome gripal com idade igual ou superior a 65 anos**, a partir de **2<sup>a</sup> feira inclusive**, até ao final da época de gripe, i.e. finais de Abril. Se não identificar nenhum caso no dia da semana referido, tente nos dias seguintes, até conseguir. Os casos podem ser seleccionados onde for mais conveniente para si, i.e., em consultas, serviços de urgência, no domicílio, atendimentos complementares, etc.

A definição de síndrome gripal é a recomendada pelo European Center for Prevention and Disease Control (ECDC):

**Grupo A + pelo menos 1 sinal ou sintoma do grupo B + pelo menos 1 sinal ou sintoma do grupo C**

### Grupo A

**Início súbito** (obrigatório)

### Grupo B

- Febre ou febrícula
- Mal-estar, debilidade, prostração
- Cefaleia
- Mialgias, dores generalizadas

### Grupo C

- Tosse
- Dor de garganta, inflamação da mucosa nasal e faríngea, sem sinais respiratórios relevantes
- Dificuldade respiratória

Se o doente estiver **a viver num lar ou residência para idosos** ou tiver **contra-indicação para a toma da vacina sazonal**, **exclua-o do estudo**, preencha a folha **branca** de recusa/exclusão e identifique outro caso de síndrome gripal.

O doente deve tomar conhecimento de que vai ser incluído neste estudo e concordar com essa participação, assinando em duplicado a folha de consentimento informado ou dando o seu consentimento verbal.

## 2. Colheita de dados

Preencha o questionário (**folha amarela, frente e verso**) que descreve a síndrome gripal (data de início dos sintomas, sintomas e sinais presentes, estado vacinal em 2010/2011 e no ano anterior, toma de antivirais e estado de saúde ou doença do indivíduo). Assinale com um X sobre o espaço ou sobre a letra adequada.

Por favor, confirme directamente com o doente ou processo clínico as respostas que vai dar.

Destaque o questionário pelo picotado, sem o cabeçalho com a identificação do utente e envie-o juntamente com o exsudado nasofaríngeo cuja colheita se descreve a seguir.

O cabeçalho com a identificação do utente deve ficar no seu caderno de questionários para futura consulta, caso haja alguma dúvida sobre o caso enviado.

### 3. Colheita de exsudado da nasofaríngea

- a) Recolha um exsudado nasofaríngeo de acordo com as instruções seguintes:
  1. Introduza a zaragatoa na narina (direita e esquerda) paralelamente ao palato e deixe nessa posição alguns segundos de forma a absorver as secreções;
  2. Introduza um pouco mais fundo na mucosa nasal (aproximadamente 2 a 3 centímetros no adulto e até o doente lacrimejar) e rode ligeiramente a zaragatoa;
  3. Retire a tampa do tubo de transporte e introduza a zaragatoa para que esta entre em contacto com a esponja existente no fundo do tubo;
  4. Pressione fortemente a parte inferior do tubo de modo a que o meio de transporte que embebe a esponja molhe o algodão da zaragatoa;
- b) Identifique o tubo com o código de caso (canto superior direito da folha amarela).
- c) A amostra biológica deve ser conservada entre 4 a 8°C até à recolha pela transportadora, para envio ao laboratório.
- d) O tubo deve ser acondicionado individualmente, vedando a sua tampa com parafilm (segue com o restante material) e introduzindo-o num saco de plástico devidamente fechado. Este saco deve ser introduzido num envelope almofadado que, por sua vez, deve ser introduzido numa das bolsas plásticas (alfapack) fornecidas.

### 4. Envio do questionário e zaragatoa para o laboratório

A rapidez no envio das zaragatoas ao laboratório constitui um dos aspectos de maior relevância para a obtenção de resultados válidos no diagnóstico. Neste sentido, solicita-se que sejam enviados o mais brevemente possível.

Os pedidos de recolha à empresa transportadora poderão ser efectuados por telefone ou por e-mail, até às 12h00, de Segunda a Quinta-feira.

- **Por telefone:** o requerente deverá ligar para a linha **707 212 707** e solicitar a recolha de encomenda no âmbito do **Programa de Vigilância da Gripe** (a chamada será encaminhada para um serviço de transporte específico para este Programa) identificando a **conta do INSA número 6226** e o seu **código 0135** seguido da localidade da recolha.
- **Por e-mail:** enviar o pedido de recolha para **[gripe@alfaloc.pt](mailto:gripe@alfaloc.pt)** indicando a **conta do INSA número 6226** e o seu **código 0135** seguido da localidade da recolha. O responsável da Alfaloc pelo registo das recolhas acusará a recepção deste e-mail no prazo máximo de 15 minutos; se o requerente não receber a confirmação da recepção do pedido de recolha neste prazo, deverá contactar o serviço de clientes pela linha **707 212 707**.

Serão disponibilizadas, nos locais de recolha, guias de transporte pré-impressas com informação do nome e morada do expedidor e do destinatário (o INSA).

Para qualquer informação adicional, por favor contactar o Laboratório Nacional de Referência para o Vírus da Gripe do Instituto Nacional de Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Tel: 217526455 ou 217519216.

